UNIVERSITY
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Development of Pressurised Hot Water Extraction for Isolation of Natural Products

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Abstract:

This thesis reports on the development of a rapid, efficient, and inexpensive pressurised hot water extraction (PHWE) technique, and its use to extract valuable natural products from plant material. Further, the use of natural products as scaffolds for divergent synthesis of known natural products and other complex molecules is described.

The PHWE method uses a standard benchtop espresso machine, and was developed and refined through the extraction of polygodial from *Tasmannia lanceolata* (Tasmanian native pepper) and shikimic acid from *Illicium verum* (Chinese star anise). The former is a relatively non-polar sesquiterpene dialdehyde containing some sensitive functionality, and the latter is a much more polar carboxylic acid. These substrates proved to be useful for method development, and provide valuable complex scaffolds for synthetic applications.

Applications of the espresso machine extraction method for rapid bioprospecting and chemotaxonomic applications were investigated through the extraction of a unique population of *T. lanceolata*, and extraction of *Drimys winteri*. This novel PHWE method was also applied to the extraction of known biologically active extracts such as that of *Momordia charantia* (bitter melon), *Eupatorium adenophorum* (Crofton weed), and *Chrysanthemum cinerariaefolium* (Dalmatian chrysanthemum, contains pyrethrins).

Additionally, the use of the PHWE technique for essential oils was investigated. Many common plants were extracted, rapidly providing enriched essential oil extracts. The extraction of eugenol and acetyleugenol from cloves was implemented into the undergraduate laboratory program as a result of this work.

The reactivity of polygodial was investigated extensively through the synthesis of some simple natural products based on this skeleton, in addition to various analogues. More complex polycyclic structures based on polygodial were also synthesised to investigate the level of stereospecificity achieved in reactions on this scaffold.

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Abbreviations:

% w/w	percentage by mass
% v/v	percentage by volume
^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon nuclear magnetic resonance
ASE	accelerated solvent extraction
atm	atmospheres
aq.	aqueous
Ar	aryl
AUD	Australian dollars
BHT	butylated hydroxytoluene, 2,6-bis(1,1-dimethylethyl)-4-methylphenol
BuLi	n-butyllithium
DMF	*N,N*-dimethylformamide
DIBAL	diisobutylaluminium hydride
dppp	1,3-Bis(diphenylphosphino)propane
ELSD	evaporative light scattering detector
GC	gas chromatography
EtOAc	ethyl acetate
EtOH	ethanol
eq	equivalent(s)
h	hours
HSQCme	heteronuclear single quantum correlation, multiplicity edited
IR	infrared
LDA	lithium diisopropylamide
MeOH	methanol
min	minutes

Ms	methanesulfonyl
MS	mass spectrometry
NBS	*N*-bromosuccinimide
NBSH	*o*-nitrobenzenesulfonyl hydrazide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
PHWE	pressurised hot water extraction
Ph	phenyl
PIDA	diacetoxyiodobenzene
pin	pinacol
ppm	parts per million
psi	pounds per square inch
rt	room temperature
s	seconds
SWE	subcritical water extraction
TBS	*tert*-butyldimethylsilyl
THF	tetrahydrofuran
TLC	thin layer chromatography
Ts	toluenesulfonyl
USD	United States dollars
UV	ultraviolet

List of Publications/Conference Presentations:

Peer Reviewed Journal Articles

Deans, R. M.; Gardiner, M. G.; Horne, J.; Hung, A. C.; Hyland, C. J. T.; Just, J.; Smith, J. A.; Yin, J. *Asian J. Org. Chem.* **2014**, *3*, 1193.

Just, J.; Deans, B. J.; Olivier, W. J.; Paull, B.; Bissember, A. C.; Smith, J. A. *Org. Lett.* **2015**, *17*, 2428.

Just, J.; Jordan, T. B.; Paull, B.; Bissember, A. C.; Smith, J. A. *Org. Biomol. Chem.* **2015**, *13*, 11200.

Just, J.; Bunton, G. L.; Deans, B. J.; Murray, N. L.; Bissember, A. C.; Smith, J. A. *J. Chem. Educ.* **2016**, *93*, 213.

Kunde, D. A.; Chong, W. C.; Nerurkar, P. V.; Ahuja, K. D. K.; Just, J.; Smith, J. A.; Guven, N.; Eri, R. D. *BMC Complement. Altern. Med.* **2017**, *1*, 4004.

Deans, B. J.; Just, J.; Chhetri, J.; Burt, L. K.; Smith, J. N.; Kilah, N. L.; de Salas, M.; Gueven, N.; Bissember, A. C.; Smith, J. A. *ChemistrySelect* **2017**, *2*, 2439.

Submitted Journal Article

De La Chapa, J.; Singha, P.; Sallaway, M.; Self, K.; Nasreldin, R.; Dasari, R.; Hart, M.; Kornienko, A.; Just, J.; Smith, J.; Bissember, A. C.; Gonzales, C. B. "Novel Polygodial Analogs P3 and P27: Efficacious Therapeutic Agents Disrupting Mitochondrial Function in Oral Squamous Cell Carcinoma" *Cell. Oncol.* **2017**, Submitted.

Book Chapters

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Ryan, J. H.; Smith, J. A.; Hyland, C.; Meyer, A. G.; Williams, C. C.; Bissember, A. C.; Just, J. Chapter 7 - Seven-Membered Rings, *Progress in Heterocyclic Chemistry*, eds. G. W. Gribble, J. A. Joule, Elsevier, 2015, vol. 27, pp. 531–573.

Conference Presentations

Just, J.; Deans, B. J.; Smith, J. A.; Bissember, A. C.; Paull, B. P.; Shellie, R. A. (September 2014) Rapid Pressurised Hot Water Extraction – Isolation of Complex Scaffolds for Medicinal Chemistry, Poster presented at Graduate Research Conference, University of Tasmania, Australia

Just, J.; Deans, B. J.; Olivier, W. J.; Bissember, A. C.; Paull, B.; Smith, J. A. (December 2014) A New Approach to Natural Products Isolation, Oral presentation at RACI National Congress, Adelaide, Australia

Just, J.; Deans, B. J.; Olivier, W. J.; Bissember, A. C.; Smith, J. A. (August 2016) The Humble Espresso Machine – Extraction of More Than Just Coffee Beans, Poster presented at Natural Products and Bioactive Compounds Gordon Research Conference, New Hampshire, USA

Chapter 1: The Significance of Natural Products

1.1 The Importance of Natural Products and Bioprospecting

Nature is, and will remain, an important source of biologically active compounds. The sheer structural diversity of natural products, and the control of chemical synthesis achieved by enzymes results in a treasure trove of compounds.[1] In 2000, it was estimated that '<10 % of the world's biodiversity has been tested for biological activity'.[2]

The natural products of today exist as a result of millions of years of biological evolution. As life has evolved, a staggering number of classes of natural products have evolved along with it, which serve as the regulators and modulators of complex biochemical processes.[3] Through the processes of evolution, the resulting set of natural products tend to possess incredible specificity with regards to the biological macromolecules with which they interact, and therefore the response and effects that they cause.[4] As natural products have evolved for specific bioactivity, many already possess the necessary features to be used as a drug. For *de novo* drug design, on the other hand, many parameters are considered in the design of the molecule, highlighted by Lipinski's 'Rule of 5', which gives parameters relating to the solubility and permeability of the molecule. These parameters are linked to the likelihood of the molecule having appropriate bioavailability as a drug.[5,6] Natural products, however, tend not to rely on passive diffusion for absorption, and so are explicitly exempt from these guidelines.[4,5,7,8]

Natural products and natural product inspired drugs are regularly reviewed in the scholarly literature, with recurring reviews on the topic. Important examples of these are the 'Natural Products as Sources of New Drugs', in the Journal of Natural Products by Gordon and Cragg with various other contributors.[9,10] The most recent of these was published in 2016, covering the period of 1981 to 2014. This review reports that over that period, an average of ~50% of the new drugs on the market are natural products, derived from natural products, or inspired by natural products. Small molecules continue to be the dominant form of new drugs on the market, with 1211 new small molecules introduced to the drug market between 1981 and 2014 out of a total of 1562 drugs. These are a combination of natural products and derivatives, as well as fully synthetic molecules, which can be natural product mimics with completely different structural properties but with the same mechanism of action as the natural product. The following

chart shows categories of small molecule new drugs on the market between 1981 and 2014. N are unmodified natural products, NB are specific mixtures of unmodified natural products, ND are derivatives of natural products, S* are fully synthetic molecules with a natural product pharmacophore, and S are fully synthetic.[11] Figure 1.1 shows that ~50 % of the new small molecule drugs on the market are related in some way to natural products, with S being the only category of compounds reported which are termed 'fully synthetic', and in no way related to natural products.

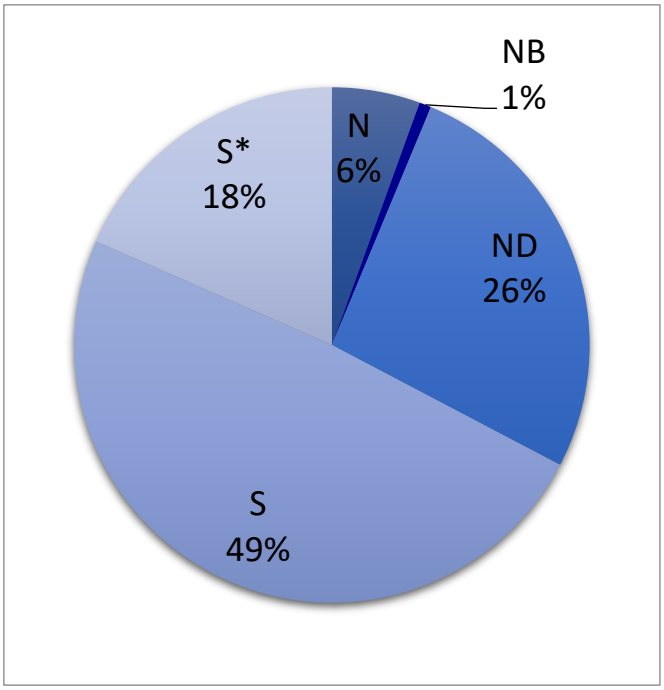

Figure 1.1 – Percentage of new drugs on the market, 1981–2014. Data from Newman and Cragg, 2016[11]

The development of more efficient testing methodologies and computing power has led to an increase in the number of molecules able to be tested for biological activity. With a combination of *in vitro* and *in silico* high throughput screening methods, and the development of huge libraries of compounds both real and modelled theoretically, it was anticipated that the number of drugs reaching the market would continue to increase.[12] This development however, led to a shift away from natural product based inspiration,[13] and the opposite was observed. One factor thought to be involved in this is non-natural products having higher levels of unwanted or dangerous side effects discovered during testing.[4] This has led to a resurgence in natural products-based drug discovery.[14,15]

There are many historical examples of natural products causing breakthroughs in medicine. For example, the antibiotic penicillin G (**1**), which was discovered in the 1920's.[16] Penicillin is produced by fungi specifically to kill bacteria, which protects the fungus from infection and means the bacteria are not competing for resources.[17] The mechanism of action of penicillin is very specific, which is part of the cause of its success as a drug. This molecule is a β-lactam antibiotic, which has a direct action on an enzyme to mediate its effect. The molecule acylates enzymes involved in the synthesis of the bacterial cell wall which cause the bacteria to be unable to develop structurally, and therefore become non-viable and die.[18] The molecule has evolved with such specificity that it only acts on certain types of bacteria and does not interact with human enzymes, leading to minimal side effects. Penicillin was a serendipitous discovery, when spores of *Penicillium* fungi landed randomly on some agar plates that had been culturing bacteria and developed zones of inhibition where the fungi were growing.[16] An advantage of using natural products from fungi or bacteria is the ease with which the production can be scaled up, as many microorganisms can be cultured easily and the exponential reproduction means that cultures grow rapidly.

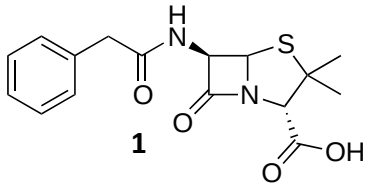

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Figure 1.2 – Penicillin G (**1**), also known as benzylpenicillin, the originally discovered penicillin molecule, showing the reactive β-lactam structural motif.

Not all natural product discoveries occur in this way, however, and may require a concerted and sometimes time- and labour-intensive exploration of nature. The process of searching for new valuable materials from natural sources is known as bioprospecting. Specifically, the World Health Organisation defines bioprospecting as "…the systematic search for and development of new sources of chemical compounds, genes, micro-organisms, macro-organisms, and other valuable products from nature."* Natural product bioprospecting can lead to new drugs on the market in three major ways:

* http://apps.who.int/medicinedocs/en/d/Jh2996e/6.3.html accessed 06/11/17

1 – The natural product is extracted and formulated into a drug directly without any modification of structure.

2 – The natural product is the drug, but the biological source is too rare or otherwise prohibitive for extraction, which means that the drug is prepared synthetically. This can be due to a number of factors - primarily the rarity of the plant, the yield of the compound from the plant, and the growth rate of the plant.[12]

3 – The natural product serves as a structurally complex starting material/precursor for the synthesis of a drug.

Concerted bioprospecting efforts have led to numerous breakthroughs in natural products chemistry. A key example of this is the discovery of natural sources of bulk quantities of steroid-based molecules, which could be used to synthesise natural steroids and analogues. Prior to this discovery the synthesis of steroids was laborious and inefficient.[19,20] The sheer structural complexity of the steroid core, with 4 fused rings and in the example of testosterone (**2**), 6 contiguous stereogenic centres, leads to a very demanding challenge, particularly regarding stereoselective synthesis.

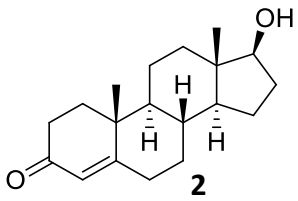

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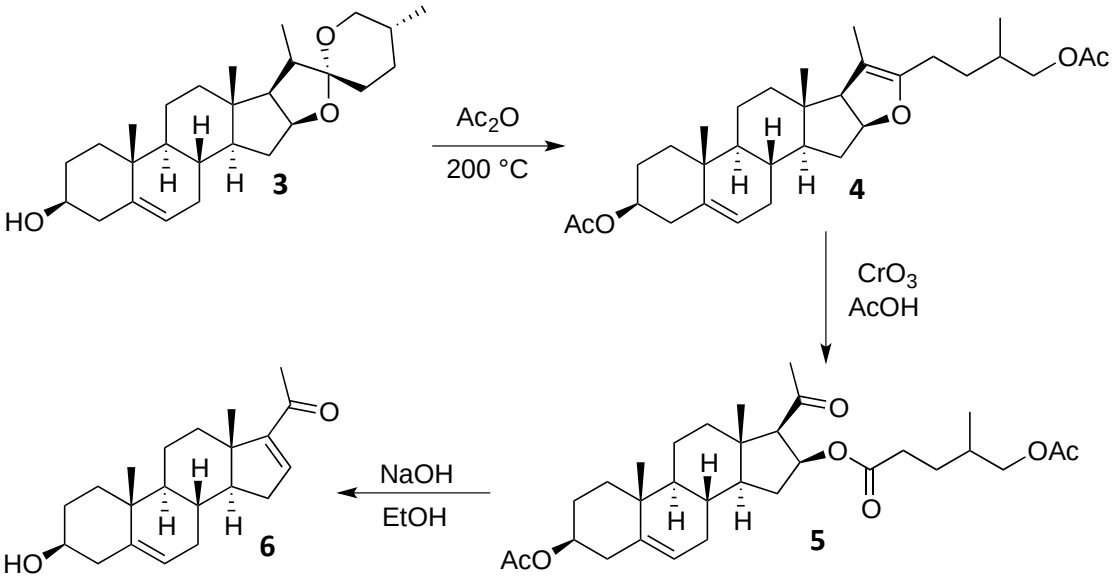
Figure 1.3 – Testosterone (**2**), a key example of a steroid hormone with 6 contiguous stereocentres.

A key step in this process was the elucidation of the true structure of plant derived sapogenins, and subsequently ways in which they could be transformed into divergent precursors for numerous steroid based molecules for anti-inflammatory and contraceptive applications.[21] The key reaction sequence in transforming plant derived sapogenins into a precursor for the divergent synthesis of medicinally useful steroids is known as the Marker degradation, after the chief researcher in the project, Russell E. Marker. The Marker degradation is a simple and high-yielding three-step process to form a readily functionalisable steroid core from a naturally derived sapogenin scaffold such as diosgenin (**3**), which may be further transformed into many important steroidal

compounds such as progesterone, cortisone, testosterone, estrone and estradiol. The first step in the Marker degradation is the ring opening of the spiroketal group using acetic anhydride at 200 °C to yield the ring-opened acetate **4**. Subsequent oxidative cleavage with chromic acid effects a further ring opening to yield compound **5**, containing the tetracyclic ring system common to all steroids. Finally, the use of sodium hydroxide in ethanol hydrolyses the resulting ester side chain, which further eliminates water to form α,β-unsaturated ketone **6**. The previously acetylated hydroxyl functionality is also restored during this reaction. The reaction sequence was applied to the numerous plant-based sapogenins sourced from various plant species.



Scheme 1.1 – Marker Degradation on diosgenin (**3**) from *Dioscoria villosa.*

A subsequent bioprospecting mission carried out through Mexico and the southern USA eventually yielded a commercially viable plant for the isolation of significant quantities of diosgenin. The Mexican yam (*Dioscoria mexicana*), an unusual caudiciform plant, became the source of kilogram quantities of diosgenin.[22] Caudiciform refers to the growth habit of the plant, where the base of the plant (caudex) forms a large bulbous mass above and below the ground (see Figure 1.4). This large mass of plant material is where the diosgenin is found. This result came from the study of ~40,000 kg of plants comprising nearly 400 different species,[23] and represents a significant achievement in bioprospecting. It was not until a plant was discovered that produced large enough quantities of this material that the medicinal steroid industry could flourish, leading to the development of a lot of new chemistry.

Figure 1.4 – Mexican yam, showing the unusual caudiciform growth habit.*

Another key historical example of the success of bioprospecting is the anti-cancer drug Paclitaxel (**7**, often referred to by a trade name, Taxol). This compound was initially isolated as an extremely active component of the extract of the Pacific yew tree (*Taxus brevifolia*). The Pacific yew tree is a very slow growing tree, which produces only minimal amounts of this compound from the bark,[24] and therefore the use of this compound directly from the source is impractical and unsustainable.

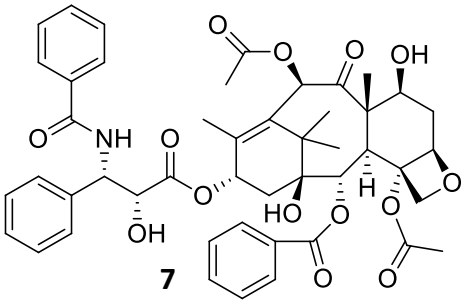


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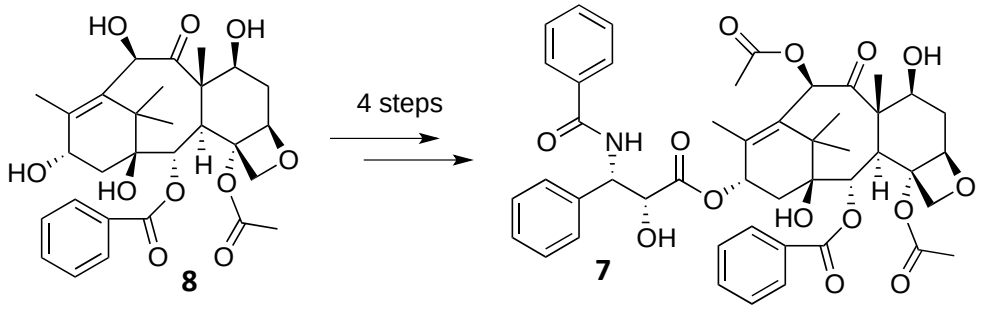
Figure 1.5 – Paclitaxel (**7**).

The first total synthesis, reported in 1994 by Holton and colleagues, demonstrates an enantioselective total synthesis in 46 linear steps.[25,26] This is one of the shorter synthetic routes, partly as the starting material is already a C_{15} compound. The total syntheses of this compound are undoubtedly huge achievements in synthetic organic chemistry, but were not sufficient to meet the demand for these biologically active molecules for clinical trials through to front line treatments. The breakthrough in being able to obtain viable quantities of paclitaxel was the discovery that a related species, *Taxus baccata*, produced

* Image by Amada44, Wikimedia Commons CC BY 3.0

significant quantities (~1 g/kg) of the natural product 10-deacetylbaccatin III (**8**), which was used for the semi-synthesis of paclitaxel in 4 steps.



Scheme 1.2 – Semi-synthesis of paclitaxel (**7**) from 10-deacetylbaccatin III (**8**)

Compound **8** represents by far the most complex portion of paclitaxel, and therefore significantly reduces the number of steps required to create the molecule. This also represents a non-linear synthesis where two parts of the molecule are isolated or synthesised independently, and subsequently joined which makes the overall process more efficient. A review by Borah and co-workers in 2007 summarises the different routes used for the synthesis and attachment of the side chain onto 10-deacetylbaccatin III.[24] This semi-synthetic approach demonstrates the unavoidable and fundamental link between these two areas of chemical research and medicinal chemistry.

Though these semi-synthetic methods were used for many years for the manufacture and supply of paclitaxel, it was still inefficient, so efforts were made towards the development of a greener method of production. Paclitaxel is now produced directly through plant cell fermentation methods further improving the viability of the industry and negating the need for chemical manipulation. This method has a number of advantages, outlined in Green Chemistry in the Pharmaceutical Industry – The Taxol® Story, Development of a Green Synthesis via Plant Cell Fermentation.[27]

The stories of diosgenin and paclitaxel are two well-known examples in which bioprospecting of plants led to the discovery of significant quantities of incredibly important materials, which in turn solved the supply problem of some very important classes of drugs. Sometimes, however, discovery of an abundant source of the natural product or a precursor in nature has proven impossible. Some examples of this are natural products from sea sponges. In the case of sponges, the natural products are often

not secondary metabolites of the sponge itself, but are often produced by symbiotic microorganisms.[28] These natural products show a huge chemodiversity and a range of different biological activities, but tend to be found in very small amounts in the organism.[29] Two important examples of natural products found in sponges are (+)–discodermolide (**9**), and the bryostatins (bryostatin I (**10**) is shown in Figure 1.6). In these two cases, the lack of abundance of the natural product or a natural product precursor meant that the supply problem needed to be solved in a different manner.

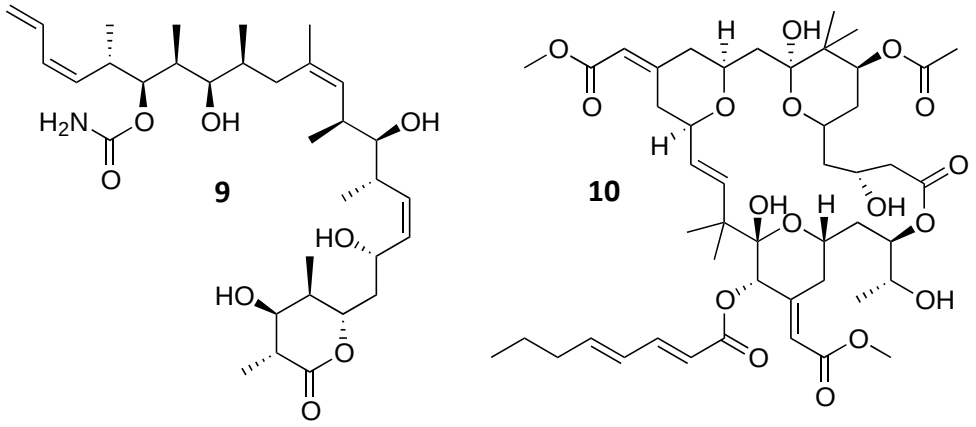


Figure 1.6 – (+)-Discodermolide (**9**) and bryostatin I (**10**), both isolated from marine organisms.

In the case of (+)-discodermolide, the original report from 1990 gave a yield of 0.002 %, following the isolation of 7 mg of the compound from 434 g of the sponge *Discodermia dissoluta*. This, in combination with the difficult collection (30 m depth) and the ethical and conservation issues surrounding collection of animal species, meant that isolation of significant quantities of this compound directly from the organism was not feasible.[30] This compound has shown significant activity in numerous anti-cancer assays, showing a similar mechanism of action to Paclitaxel.[31] In addition, some paclitaxel-resistant cancer cell lines are susceptible to (+)-discodermolide.[32] For these reasons, the development of a synthetic route to this important compound was desired. Syntheses and improvements were developed over the course of ~14 years before significant quantities could be produced. Numerous research groups published syntheses during this time, notably Smith's gram scale synthesis reported in 1999,[33] with subsequent improvements published over time.[34] The chemical company Novartis used elements of Smith's approach, in combination with the work of Paterson[31] to complete a 60 g synthesis of this

compound.[35] In spite of the overall yield being <1 % for this synthesis compared with overall yields of around 7–11 % for other reported syntheses, the sheer scale of the reactions represented a significant achievement in chemical process development.

Bryostatin I (**10**) was the first of 20 similar structures discovered.[36] This molecule has been investigated for its therapeutic potential in the treatment of HIV, cancer, and Alzheimer's disease, but maintaining adequate supply has proven a significant challenge.[37] Originally discovered in the 1960's, the structure of bryostatin I was elucidated and reported in 1982. Isolated from the nudibranch *Bugula neritina*, this compound exists in even lower quantities than (+)-discodermolide, with a report of 500 kg of the creatures providing a mere 120 mg of the compound, representing a staggeringly small yield of 2.4 x 10^{-5} %.[38] Collection in a separate instance of 14 tons of these creatures led to a yield of 18 g, representing a much higher but still unsustainable yield of 1.4 x 10^{-4} %.[39] Only in 2017, has the supply problem been convincingly solved. Wender and co-workers have published a scalable synthesis of bryostatin 1.[37] Their reaction sequence comprises 29 total steps, a longest linear sequence of 19 steps, >80% average yield, and an overall yield of 4.8 %.

These examples highlight the fundamental and interconnected nature of bioprospecting, as well as organic synthesis in the discovery, isolation and ongoing supply of important natural products of medicinal significance. For the above reasons, natural products research will continue to be hugely important for the discovery of novel biologically active molecules. It is therefore desirable that efficient methods of extraction and isolation of natural products continue to be developed to facilitate the investigation of the remaining ~90% of unexplored natural sources. A key focus of this thesis concerns the development of a novel, simple, rapid, and cost-effective method for plant extraction based on Pressurised Hot Water Extraction (PHWE), which uses a standard benchtop espresso machine.

1.2 Tasmania and Australia as Significant Bioprospecting Sites

As highlighted by the examples provided above, plants are an important class of organisms in natural product discovery. Indeed, plants were used by the earliest humans as remedies for various ailments with varying levels of success. Many of these traditional medicinal plants have been investigated and the active principles identified.[40] It is recognised that Australia is a location of plant endemism* of international significance, with a large number of plant species totally unique to the continent.[15,41] An example of bioprospecting of Australia's unique flora is the natural product tigilanol tiglate (**11**). This compound is only found in the species *Fontainea picrosperma* (Euphorbiaceae), which is endemic to Queensland, Australia. Through a bioactivity guided bioprospecting effort, this compound was isolated and investigated in pre-clinical trials, showing promising activity against certain kinds of tumours.[42]

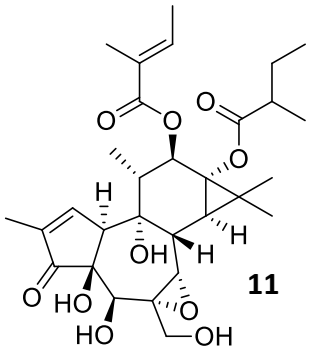


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Figure 1.7 – Tigilanol tiglate, isolated from *F. picrosperma*, endemic to Queensland, Australia.

Australia has many areas of highly unique endemic species, and Tasmania is recognised as one of these areas.[41,43] Indeed, Tasmania is known to be home to many relictual plant species, being the only surviving species from formerly large groups of plants.[44,45] Additionally, many of these Tasmanian species have not been examined for their chemical content.[46] Due to all of these factors, there is a wealth of biodiversity unique to Tasmania that merits investigation. To this end, development of an inexpensive and efficient bioprospecting method utilising a modern and green technique is of great significance.

* A plant species is endemic to a region if it only exists in the wild in that particular region. This is a broad term, without specific definition of the size of that area.

1.3 Traditional Extraction Methods

A review from 2013 lists the following three techniques as being the major traditional methods of extraction from plants: maceration, Soxhlet extraction and hydrodistillation.[47] All techniques have various benefits, as well as drawbacks. When new extraction techniques or modifications of existing techniques are developed, the reports typically include a comparison to, or benchmarking against one or more of these traditional extraction methods in determining efficiency.

1.3.1 Maceration

Maceration involves soaking the material containing the target compounds in a solvent, and is operationally the simplest technique. Maceration can be performed with or without stirring/agitation, and can be conducted at any temperature with the usual considerations of solvent boiling point and target analyte stability. Due to the simplicity of the technique, no specialised glassware or other equipment is required for the extraction. This technique suffers from drawbacks which lead to a loss of efficiency including relatively long extraction time, no recycling or flow of solvent leading to solvent saturation, and little selectivity in extraction.[48] Macerations typically suffer from low yields and difficulty in purification due to the extraction of plant pigments.[49]

1.3.2 Soxhlet Extraction

Soxhlet extraction is similar to maceration in that the material to be extracted is soaked in a solvent. The primary difference between these two techniques however is that Soxhlet extraction uses a semi-continuous extraction process where pure solvent is distilled into the sample from a solvent reservoir, where effectively a maceration takes place until a set volume is reached. The solvent then drains through a siphon mechanism back into the solvent reservoir and the process is repeated. In this way, the concentration of extract continues to increase, as fresh solvent is recycled over and over. This method usually shows a very high overall extraction efficiency but suffers from specialised apparatus requirements, particularly for large-scale extractions. The long extraction time can also lead to degradation of thermolabile substances.[50]

1.3.3 Hydrodistillation

Hydrodistillation is the immersion of material into water, followed by distillation. This process extracts largely the essential oil components of the material within a certain vapour pressure range, leaving everything else behind. This method shows a high selectivity towards the types of compounds which are extracted leading to a generally very clean extract, but can only be used for compounds that meet the criteria. This method has continued utility in the perfume, flavour, and essential oils industries[47] but due to the narrow scope is not a typical technique for more general bioprospecting. Drawbacks include narrow extraction scope, relatively long distillation time, and a sustained high temperature of the sample material.[47]

1.3.4 Modifications to Traditional Methods

There are some modifications made to traditional extraction methods designed to increase their efficiency. These include ultrasound assisted extraction,[51-53] microwave assisted extraction,[54-56] pulsed-electric field extraction and enzyme assisted extraction.[47] These methods involve a physical or chemical mechanism to free up the desired components within the plant matrix such that they are more readily extracted into the extraction solvent.

1.4 Modern Extraction Methods

Trends in green chemistry have pushed the focus towards the use of 'green' solvents. A review of the 'greenness' of solvents from 2007 states that from an environmental perspective, simple alcohols and alkanes are favourable solvents to use, along with water.[57] As traditional plant extraction methods often use large volumes of toxic and environmentally unfriendly solvents,[58] modern techniques have been developed to increase the efficiency, repeatability and overall 'greenness' of extractions by reducing solvent consumption, or utilising alternative solvents. Two prominent examples of these methods are supercritical fluid extraction, and pressurised hot water extraction.

1.4.1 Supercritical Fluid Extraction

Supercritical fluid extraction is typically undertaken using solvents which are gases at atmospheric pressure and temperature. These include CO_2, propane, butane and ethylene, with CO_2 being by far the most commonly employed.[59] Due to the pressures

required to keep these solvents in the supercritical fluid state (for CO_2, the critical point is 73.8 bar and 31.1 °C.)[60], this technique requires highly specialised equipment. A major advantage of this technique is that the solvent evaporates from the resulting extract rapidly upon returning to atmospheric pressure. This technique has a major industrial application in the decaffeination of tea and coffee.[59]

In spite of the advantages of this modern technique, more modern does not always mean more efficient for every example. This is highlighted in the extraction of thujones from *Salvia officinalis*, where a direct comparison of hydrodistillation and supercritical fluid extraction showed hydrodistillation to be a more efficient extraction technique.[61] Another example of this is the extraction of phenolics from *Echinacea purpurea*, where a Soxhlet extraction was found to be vastly superior in overall extraction yield compared to supercritical fluids extraction with CO_2, microwave assisted extraction with EtOH, and pressurised liquid extraction with water at 60 °C.[62] The Soxhlet extraction, however, did use large quantities of solvent, and took a much longer time than the other techniques.

1.4.2 Pressurised Hot Water Extraction

Pressurised Hot Water Extraction (PHWE) (also known as SWE - subcritical water extraction)[59] was first reported in 1994 with the investigation of sub- and super-critical water as a solvent for extracting organic material from environmental solids,[63] and remains a popular method of extraction of compounds from complex matrices for analytical purposes.[64-67] Its popularity is likely in part due to its more 'green' nature from a solvent perspective,[68] and its demonstrated time efficiency compared with more traditional techniques.[69] There remains, however, room for the application of this method to the extraction of natural products on preparative and semi-preparative scale. Teo and co-workers published a review on PHWE in 2010, and state in their conclusion "PHWE is a feasible green extraction method to be exploited in future technologies for more analytes to be used on a bigger scale." PHWE is preferred for food-type applications as toxic solvents are avoided during the process.[70]

PHWE is typically undertaken using custom systems[64,71-73] composed of the following components – solvent reservoir, solvent pump, oven, heating coil, sample compartment, backpressure regulator, and extract reservoir.[63,71,74] The majority of reports that feature

these apparatus are for analytical scale applications, rather than isolation of compounds on a semi-preparative to preparative scale.[66]

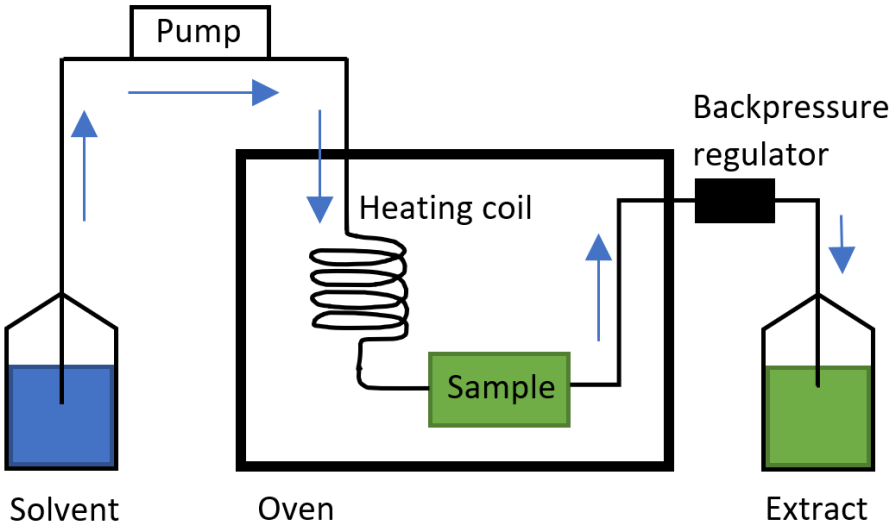

Figure 1.8 – Schematic of typical PHWE apparatus, adapted from Ong *et al.* 2010.[66]

1.5 Household Espresso Machine for PHWE

1.5.1 Espresso Machines for Coffee Extraction

PHWE is undertaken every day, all over the world, in homes, workplaces, cafes, restaurants, etc. for the creation of espresso coffee. Typically, espresso is created using the ubiquitous espresso machine, which is relatively inexpensive and readily available. Coffee is one of the most popular beverages in the world, and additionally is one of the most traded commodities in the world.[66a] Due to this popularity, extensive research into the biologically active components of coffee, as well as research into the flavour profile of coffee has been undertaken.[66b,66c] As a result, the parameters of extraction in the espresso machine have been honed and optimised over time to result in today's espresso machine.

Espresso coffee is an aqueous mixture of compounds, extracted from coffee beans, primarily of the species *Coffea arabica*. A large number of compounds are extracted from these beans, which represent a number of different molecule types over a large range of polarities from very polar substances such as inorganic salts and sugars, through to relatively non-polar substances such as waxes and oils.[66a] The most popular and well-known compound extracted from *C. arabica* is, of course, caffeine. The ability of

pressurised hot water to extract such a diverse range of compounds can be explained by the mechanisms of PHWE extraction, which involve dissolution of compounds into the water, as well as the physical extraction of less polar components.[66]

1.5.2 Similarities with Standard PHWE Equipment

The essential elements of a PHWE apparatus are contained within the espresso machine. However, there are some notable differences between typical PHWE apparatus and the espresso machine. Firstly, the espresso machine lacks a heated compartment, instead having a small heated block for heating the solvent. Secondly, the pressure is controlled by a combination of the nature and packing of the sample material, and the restriction of flow through the sample basket due to the small holes in the filter. The sample baskets are described in more detail in section 1.5.3.

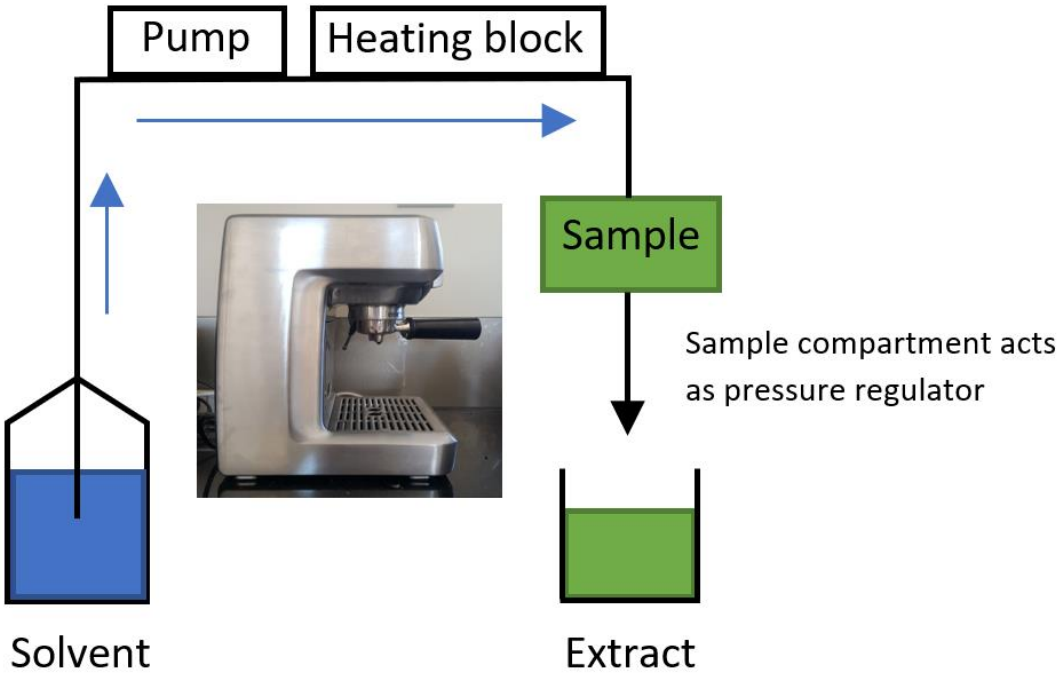

Figure 1.9 – Schematic of the espresso machine used in this study.

A major advantage of using an espresso machine is that they are readily available and inexpensive. They are also small and portable, and have a small footprint. Further, the sample compartment can contain around 10 to 20 g of material per run, which combined with the rapid nature of the extraction allows for efficient scale-up of extraction.[46,75-77] A disadvantage, however, is that pressure and temperature cannot be manually controlled, with average espresso machine pressure being 9 bar, and temperature around 92 °C.[78,79]

The temperature remains largely constant, whereas the actual extraction pressure is determined by the packing of the sample into the coffee machine and the sample basket chosen. In a comparison for the specific purpose of producing espresso, it was shown that the chemical composition of the coffee produced was essentially the same regardless of whether pressure and temperature were constant, or whether these two variables fluctuated slightly during the extraction.[79]

One significant difference of using an espresso machine for PHWE, compared with producing espresso coffee, is that with coffee the optimal shot volume is 25 mL,[79] which is designed for non-exhaustive extraction of components to give the ideal flavor profile. This is in contrast to the aim of natural products extraction, which is to achieve exhaustive extraction of the desired compounds.

Additionally, given the scale of the coffee industry internationally, there may be opportunity to scale up PHWE using apparatus used for coffee extraction with already available machinery, rather than relying on *de novo* design of upscaled equipment.

1.5.3 Espresso Machine Characteristics

The espresso machine used, a Breville model 800ES, operates at conditions of approximately 9 bar pressure, which is variable depending on the sample holder basket used, and the characteristics of the plant material extracted. This model does not have a temperature gauge, so a separate machine was modified with a thermocouple and control box to allow monitoring and adjustment of the extraction temperature. Monitoring of the temperature for various extractions revealed that as the extraction continues, the temperature drops. This is because much larger volumes of extraction solvent are taken than an espresso machine was designed for (100–200 mL vs 30–60 mL), which cools the heating block as it flows through. Due to this, a plant extract is taken as a sum of smaller volumes (2 x 100 mL vs 200 mL), to allow for the instrument to maintain the correct extraction temperature.

Figure 1.10 – Espresso machine modified with thermocouple and control box.

There are two types of sample baskets (Figure 1.11), which differ in their innate ability to increase the pressure of the extraction. There is a single–walled basket which has ~550 holes (~310 µm diameter) for solvent extract to pass through, and there is a double walled basket which has two layers. The top layer of the double walled basket contains ~330 holes (~300 µm diameter) which lead to a small cavity, with a second wall which only has one hole (~560 µm diameter). The double–walled basket leads to a higher innate pressure as all the solvent is forced through the single ~560 µm diameter hole. This basket is used for coarser materials to increase the pressure of the extraction. In contrast, the single-walled basket is used when fine plant material causes continual blockage of the machine upon extraction. These are also known as the pressurised (double-walled) and non-pressurised (single-walled) baskets.

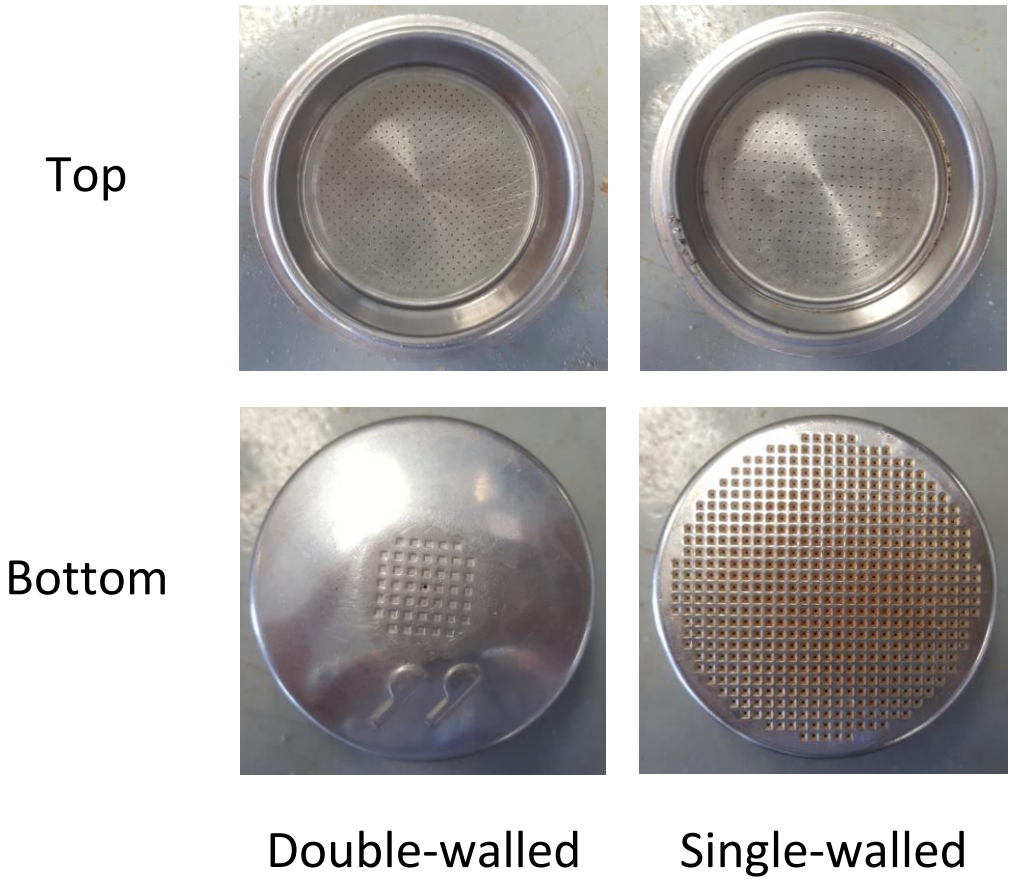

Figure 1.11 – Espresso machine sample baskets

The default basket that typically accompanies inexpensive espresso machines is the pressurised basket, and this was used for all method development. Subsequently during investigations into applications and scope of the method, the other type of basket was trialled. Typically, as pressure is a desired factor of extractions, the non-pressurised basket was only used when the pressurised basket was deemed inappropriate due to excess pressure which manifests in a very low flow rate or repeated blockages.

1.5.4 PHWE Solvent Considerations

The properties of the extraction solvent for the polarity of the compounds of interest may be modified by the addition of EtOH, which has been shown previously to influence extraction efficiency in PHWE.[67] Indeed, EtOH has been also used as a co-solvent in supercritical fluid extraction with CO_2.[62] EtOH is also more compatible with the plumbing of the espresso machine, whereas another solvent like dichloromethane is much more likely to cause problems with parts and tubing of the machine.

Solvent polarity correlates with the dielectric constant of the solvent, which in the espresso machine is dependent on temperature and proportion of EtOH, as the low pressure of the espresso machine does not influence this parameter.[80,81] The dielectric constant of water at 92 °C is ~56, and for a 35 % EtOH/water mix (v/v) at 92 °C it is ~44. This is equivalent to pure water at approximately 150 °C.[82] This allows the PHWE method to extract a broader range of compounds, without increasing the risk of compound degradation due to high temperature.[83-85] While pressure does not have an effect on the dielectric constant within this range, it does play a major role in the efficiency of the extraction. Higher pressure means that the solvent is forced into tighter spaces in the sample matrix, which may not be reached by the extraction solvent under atmospheric pressure.[63,66,86]

1.6 Summary

All of the discussed extraction methodologies have their advantages and disadvantages. The espresso machine PHWE method is no different in this regard. Development of this method is not intended to replace any established method for natural product extraction. Instead, the aim is to provide an inexpensive, efficient and green complementary method. There will be examples for which this method may not be appropriate and another method will be more suitable. Indeed, an example from our research group published recently showed the difference between a simple maceration in ether compared to espresso machine PHWE for the extraction of *ent*-labdane diterpenoids from *Dodonaea viscosa*, with the maceration proving a more effective technique for some of the less polar metabolites of the plant.[87] The physical structure of the plant material and the location of the compounds within the plant matrix meant that a simple room temperature maceration with diethyl ether was a more efficient technique. Subsequently, further investigation of PHWE of *D. viscosa* with the espresso machine was undertaken and the more polar compounds were the major components isolated.[88] This demonstrates the complementarity of the espresso machine PHWE method with other classical extraction methods. It is anticipated that development of this method will provide a unique, cost-effective, and efficient tool for the bioprospecting of Tasmania's unique flora, and for the isolation of valuable natural products.

1.7 Project Aims

To determine the viability of using an unmodified benchtop espresso machine for PHWE of natural products from plant material. (Chapter 2)

To investigate the scope and applications of the method through application to various plant species. (Chapter 3)

To utilise the natural product polygodial as a precursor for the divergent synthesis of known natural products and analogues, as well as other complex molecules. (Chapter 4)

Chapter 2: PHWE Method Development

2.1 Overview

In this chapter, the viability of using an unmodified benchtop espresso machine for PHWE of natural products from plant material was evaluated. The method was developed through the extraction of polygodial from Tasmanian native pepper (*Tasmannia lanceolata*), shikimic acid from Chinese star anise (*Illicium verum*) and coumarins from three species of *Correa*. These examples represent compounds featuring different polarities and functional groups with varying sensitivities, which in combination provide proof-of-concept for the use of a benchtop espresso machine for the rapid extraction of a wide range of valuable natural products by PHWE.

2.2 Polygodial from *Tasmannia lanceolata*

2.2.1 Background

For the initial development and characterisation of the PHWE method the extraction of the sesquiterpene dialdehyde polygodial (**12**) from *Tasmannia lanceolata* ((Poir.) A.C.Sm.) (Winteraceae, Tasmanian native pepper) was investigated. *T. lanceolata* is commonly found in the wild, in gardens, and grown commercially in Tasmania primarily for culinary applications.

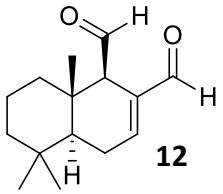

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Figure 2.1 – Polygodial (**12**), showing absolute stereochemistry

Polygodial is found in plants in the Winteraceae,[89] Polygonaceae[90] and Cannellaceae[91] families, and has also been found in marine sponges and molluscs.[92,93] Polygodial and derivatives have demonstrated a variety of biological activity, including activity as an antifouling biocide,[94] a deterrent and anti-feedant for various insect species,[92,95,96] as well as showing anti-leishmanial, anti-trypanosomal,[97,98] anti-fungal[91,99,100] and anti-bacterial[101] activity. The leaves of *T. lanceolata* are known to contain polygodial in varying quantities in wild samples of between 0.1 and >3 % (w/w).[102] *T. lanceolata* has

been heavily studied for its phytochemistry and chemotherapeutic potential, as reviewed by Cock in 2014.[103]

Polygodial is a potentially useful scaffold for synthesis owing to the two distinct aldehydes, and numerous natural products with a similar structure, but is expensive to source commercially.* Further, polygodial contains an epimerisable stereogenic centre, and is therefore potentially susceptible to isomerisation during extraction. In addition, this molecule is relatively non-polar, therefore aqueous solvent mixtures would not be the typical first choice for extraction. These combined features make polygodial from *T. lanceolata* an excellent target molecule for PHWE method development.

Previous reports detail the isolation of gram-scale quantities of polygodial, however, the methods were not efficient. In one example, 1.73 g (0.23% w/w) of polygodial was isolated from 660 g of *T. lanceolata*, with 19 L of hexanes used for the initial extraction.[104] Another more recent report describes the extraction of 896 g of leaf material from *Drimys brasiliensis* (Winteraceae) with an unspecified amount of hexanes, and 2 chromatographic steps to yield 1.09 g (0.12% w/w) of polygodial.[89] Multiple chromatographic steps for the isolation of this compound have been frequently reported.[89,98,105,106]

2.2.2 Preliminary Extraction Conditions

Initial test conditions using the benchtop espresso machine were ~15 g of dried plant material, 35% v/v EtOH:H_2O in the espresso machine, followed by liquid-liquid extraction of the ensuing mixture with CH_2Cl_2. The PHWE solvent composition was chosen due to high content of H_2O and lower flammability, with the polarity considerations discussed in Chapter 1.

*USD$162 for 10 mg as at 25/10/17 from Sigma–Aldrich.

Figure 2.2 – Espresso machine extraction of *T. lanceolata* (left), leaf material packed into the sample basket (right).

Finely ground plant material* was packed into the sample basket of the espresso machine in the same way as for ground coffee (see Figure 2.2, above). The material was extracted using the EtOH:H_2O solvent mixture, and the extract was cooled in an ice bath to limit any thermal degradation that may occur post-extraction. The PHWE extraction was carried out in 2 x 100 mL fractions with ~1 min in between to allow the espresso machine to maintain temperature, as they are typically designed for up to ~60 mL extraction volume. In this way, 15 g of plant material was extracted by this method within 2 minutes.

The resulting aqueous extract was then extracted by a liquid-liquid extraction with an organic solvent to obtain the crude polygodial extract. Dichloromethane was initially used as the secondary extraction solvent. The key signals in the ^{1}H NMR spectrum of the resulting crude extract are the aldehyde signals at 9.50 and 9.43 ppm, as well as the alkenyl signal at 7.12 ppm, indicating polygodial (**12**) as the major component of the extract.

* Plant material provided by Diemen Pepper, Tasmania, Australia, www.diemenpepper.com

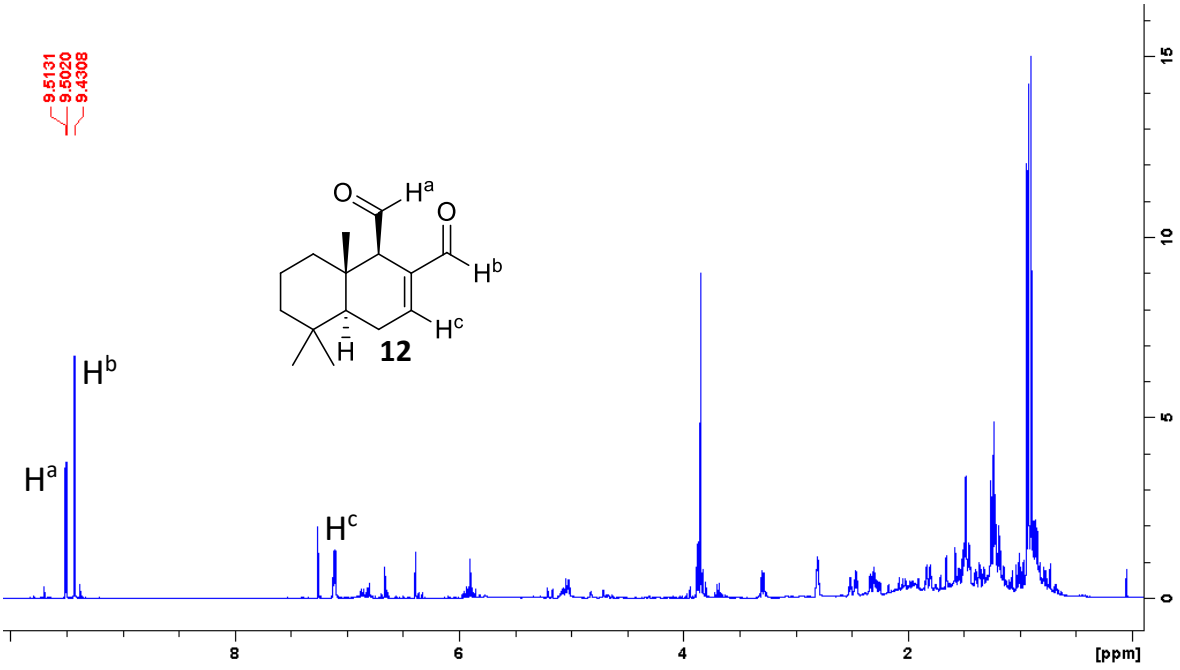


Figure 2.3 – ^{1}H NMR spectrum of commercial *T. lanceolata* crop dichloromethane extract.

Column chromatography of this initial sample provided 77 mg of polygodial from 15 g of plant material (0.5 % w/w) within a matter of hours. This was a significantly higher yield than many of the previously reported isolations, which provided a proof-of-concept that the espresso machine is a viable instrument for the PWHE of polygodial from *T. lanceolata*. Following from the success these successful initial trials under unoptimised conditions, various key parameters and variables were investigated in an effort to maximise yields.

2.2.3 Sample Pressure

As discussed in Chapter 1, the espresso machine used does not have a pressure gauge or an extraction pressure setting. However, pressure can be qualitatively determined by the user based on the flow-rate from the machine. During initial investigations, it was noted that the espresso machine was susceptible to blockage if the sample was too tightly packed or too finely ground. This was overcome in two different ways – the addition of sand (2 g sand with 15 g of dried plant material) to a tightly packed sample, and packing the sample less tightly. Although both measures alleviated the blockage of the machine, a significantly higher yield of crude extract (3.3 vs 1.7 % w/w) was obtained when the

sample was packed tightly with the addition of sand, providing evidence that pressure does have an effect on the extraction process.

2.2.4 PHWE Solvent Concentration Optimisation

The effect of the solvent composition in the espresso machine was investigated to determine the effect of ethanol concentration on the extraction yield. Solvent compositions of 0, 15, 25, and 35% v/v EtOH:H_2O were tested, and the respective masses of the crude organic soluble extracts obtained were compared (Table 2.1). As the concentration of ethanol increased, the mass of the crude extract increased, with the highest yield obtained from extraction with 35 % EtOH (v/v). Pleasingly, the ^{1}H NMR spectrum of this crude polygodial extract indicated the highest proportion of polygodial. Concentrations of ethanol higher than this were not attempted due to the potential hazards arising from the use of flammable solvents. The extract from the 35 % v/v EtOH:H_2O extraction was purified by flash column chromatography. In a single chromatographic run, pure polygodial was obtained in an average of 0.71 % w/w from a crude extract yield of 3.9 % w/w.

Entry	EtOH %v/v	Technique	Crude mass, % yield w/w	Purified polygodial, % yield w/w
1	0	PHWE	87 mg, 0.58 %	not purified[a]
2	15	PHWE	218 mg, 1.45 %	not purified[a]
3	25	PHWE	341 mg, 2.27 %	not purified[a]
4	35	PHWE	586 mg, 3.91 %	106 mg, 0.71 %

Table 2.1 – Crude *T. lanceolata* extract obtained from varying primary solvent composition in PHWE (average yields of two extractions). [a] – The crude mass obtained was sufficient information to compare the extractions at different solvent concentrations.

Figure 2.4 – PHWE extract of *T. lanceolata* (left) and the resulting isolated polygodial (right).

2.2.5 PHWE Solvent Volume Optimisation

The volume of solvent through the espresso machine required to achieve exhaustive or near exhaustive extraction of the desired compound was then investigated. Using all previously defined parameters, an extraction was carried out where three 100 mL fractions were taken from the espresso machine during extraction of *T. lanceolata* (15 g) and extracted individually with CH_2Cl_2. The yields from the three fractions were 355, 156, and 31 mg respectively. Additionally, the ^{1}H NMR spectrum showed that the first fraction contained the highest proportion of polygodial. This experiment provides evidence that 200 mL of extraction solvent was sufficient for the efficient extraction of polygodial from *T. lanceolata*.

2.2.6 Comparison to Maceration

To provide validation for the applicability of this novel PHWE method to *T. lanceolata*, a comparison was undertaken to a more basic and more traditional extraction technique – maceration. Using the same solvent, 35% v/v EtOH:H_2O, *T. lanceolata* was subjected to maceration for 24 h at ambient temperature, and in a separate experiment, for 1 h at reflux. In both cases, analysis of the ^{1}H NMR spectra showed extracts qualitatively far less enriched in polygodial than the PHWE extract, with a significantly reduced crude yield compared to PHWE. A maceration was also undertaken with CH_2Cl_2, which provided a significantly increased crude yield, however, analysis of the ^{1}H NMR spectral data for this extract showed a significantly diminished proportion of polygodial compared with the PHWE extract. Column chromatography of this material provided polygodial as a green oil (Figure 2.5), which showed residual impurities by ^{1}H NMR spectroscopic analysis. This is consistent with the previously mentioned reports of multiple chromatographic steps

required for purification of polygodial. The differing level of extraction of the green pigment components during extraction can be seen in Figure 2.6, which shows the colour of the plant material after PHWE extraction, and after maceration. This comparison provides evidence for the PHWE method being superior in efficiency and extract quality, compared with the basic technique of maceration. Further, the insoluble plant material proved more difficult to remove following maceration, which highlights another advantage this new PHWE method, in that the espresso machine also acts as a filter to exclude solid plant materials from the extract.

Entry	EtOH %v/v	Technique	Crude mass, % yield w/w	Purified polygodial, % yield w/w
Table 2.1, Entry 4	35	PHWE	586 mg, 3.91 %[a]	106 mg, 0.71 %[a]
1	35	Reflux maceration, 1 h	180 mg, 1.20 %	-
2	35	rt maceration, 24 h	210 mg, 1.40 %	-
3	CH_2Cl_2	rt maceration, 24 h	1100 mg, 7.30 %	165 mg, 1.10 %[b]

Table 2.2 – Crude *T. lanceolata* extract comparison with maceration. [a] - average of two runs, [b] - the resulting material following flash column chromatography was dark green and contained impurities.

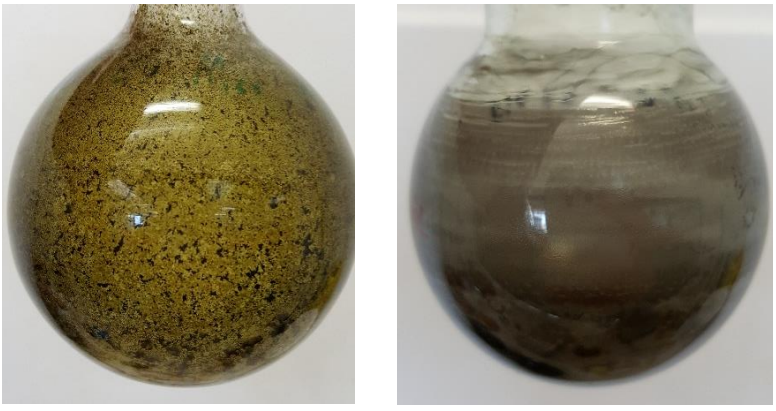


Figure 2.5 – CH_2Cl_2 maceration extract of *T. lanceolata* (left) and the resulting isolated polygodial sample (right).

Figure 2.6 – *T. lanceolata* plant material after espresso machine PHWE (left) and CH_2Cl_2 maceration (right).

2.2.7 Temperature and Pressure Optimisation through Accelerated Solvent Extractor PHWE

Following espresso machine PHWE method development, a study was undertaken using a Dionex ASE200 accelerated solvent extractor (ASE) system. As pressure and temperature cannot be programmed using the espresso machine, this system provided an alternative means of investigating these parameters. The accelerated solvent extractor is used for pressurised liquid extraction, and represented the closest available comparison to espresso machine PHWE to investigate extraction performance and yield.

The lowest pressure the ASE can be set to is 500 psi (~35 bar), so values of 500 psi and 1500 psi (~100 bar) were used to investigate if a higher pressure changed the efficiency of the extraction. Temperatures of 70, 90, 110 and 130 °C, and solvent concentrations of 15, 25 and 35 % EtOH:H_2O (v/v) were chosen. A single run was also undertaken with neat water as the extraction solvent at 150 °C to gauge the effect of increased temperature on the extraction. This instrument differs from the espresso machine in a few different ways. The espresso machine is a flow system, whereas the ASE uses static extraction. For these experiments, the system was set up to have 2 x 10 min static extractions, with solvent flushing in between. This lowers the overall extraction volume used, but increases the time of the extraction and therefore the amount of time the compounds in the extract are subjected to elevated temperature. The sample compartments of the instrument could each only contain about half the mass of *T. lanceolata* leaf material as the espresso machine (~7 g), but 24 samples could be set up and extracted automatically.

In preparation for the study, a method was developed for the quantitation of polygodial in an extract, without requiring isolation of the polygodial. This involved the use of a standard added to the crude extract, which could be compared to the amount of polygodial in the sample by integration of the relevant peaks in the ^{1}H NMR spectrum. The standard chosen was 1,3,5-trimethoxybenzene (**13**) because it is a stable, non-hygroscopic solid with a very simple ^{1}H NMR spectrum. The ^{1}H spectrum for this compound is simply a singlet integrating for the three aromatic protons at 6.08 ppm, and a singlet integrating for the nine methoxy protons at 3.76 ppm. The singlet at 6.08 was used as the signal for quantitation, compared with the integration of the aldehyde signals for polygodial.

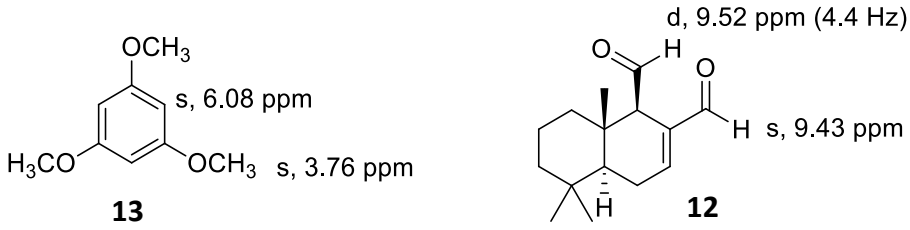


Figure 2.7 – The quantitation standard used, 1,3,5-trimethoxybenzene, polygodial, and the signals used for quantitative ^{1}H NMR spectroscopic analysis of the crude extract.

Initial investigation by ^{1}H NMR spectroscopy of this standard in a sample of crude *T. lanceolata* extract showed that the default ^{1}H NMR program on the 400 MHz instrument at the University of Tasmania was not quantitative. Quantitation relies on total relaxation of the protons after a pulse in the instrument, which occurs at different rates depending on the chemical environment of the proton.[107,108] For these experiments to be quantitative a 1:1 integration of the aldehydes with respect to the alkene signal in polygodial, and a 3:9 integration of the aromatic protons to the methoxy protons within the standard is desired. Initial experiments showed that these integrations were not matching these expectations (Figure 2.8) and could not therefore be used in quantitative experiments.

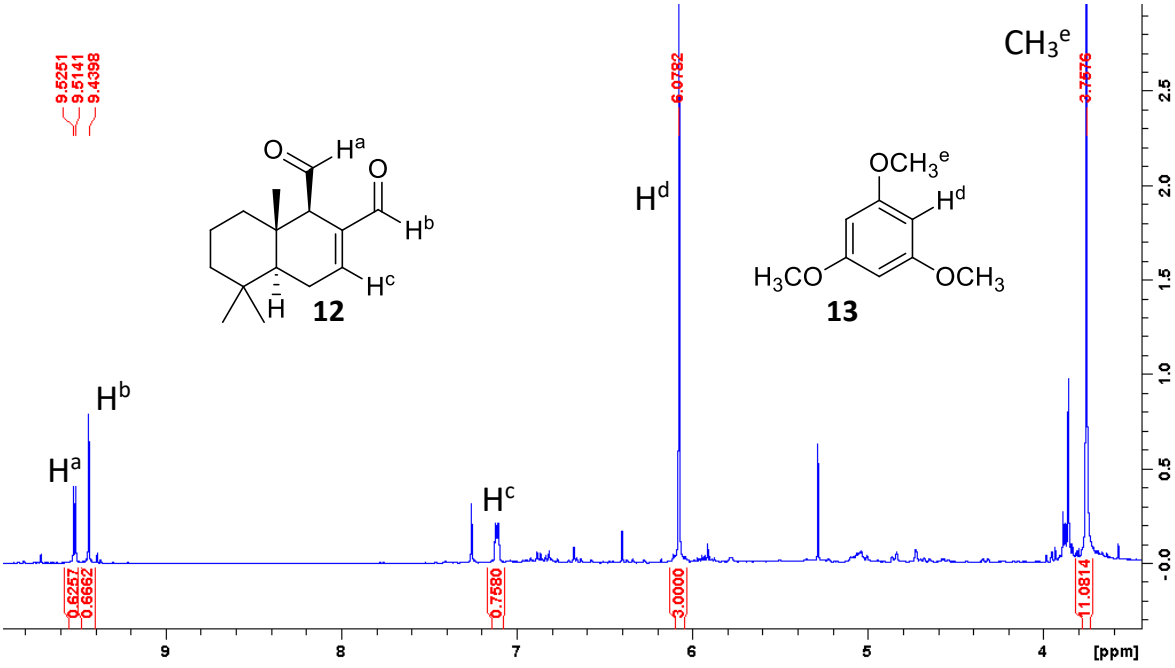


Figure 2.8 – ^{1}H NMR spectrum of a crude *T. lanceolata* extract, containing 1,3,5-trimethoxybenzene as the internal quantitation standard. Recorded using default acquisition parameters.

Relaxation is influenced primarily by the angle of the pulse, and the relaxation delay. A lower angle pulse means that the protons relax and realign with the magnetic field more quickly, and a longer relaxation delay gives more time for this to occur.[78,107] The default program on the Bruker 400MHz instrument has a 90 ° pulse angle and a 5 s relaxation delay. For quantitation, the pulse width was reduced to 30 °, and the relaxation delay increased to 10 s. Figure 2.9 shows a mixture of polygodial (**12**) and the NMR standard **13** recorded using the default parameters. It can be seen that the integrations within each molecule are not quantitative. Figure 2.10 shows the result of changing the parameters to a 30 ° pulse width and a 10 s relaxation delay, where the protons within each molecule show the appropriate integration ratio required for a quantitative analysis.

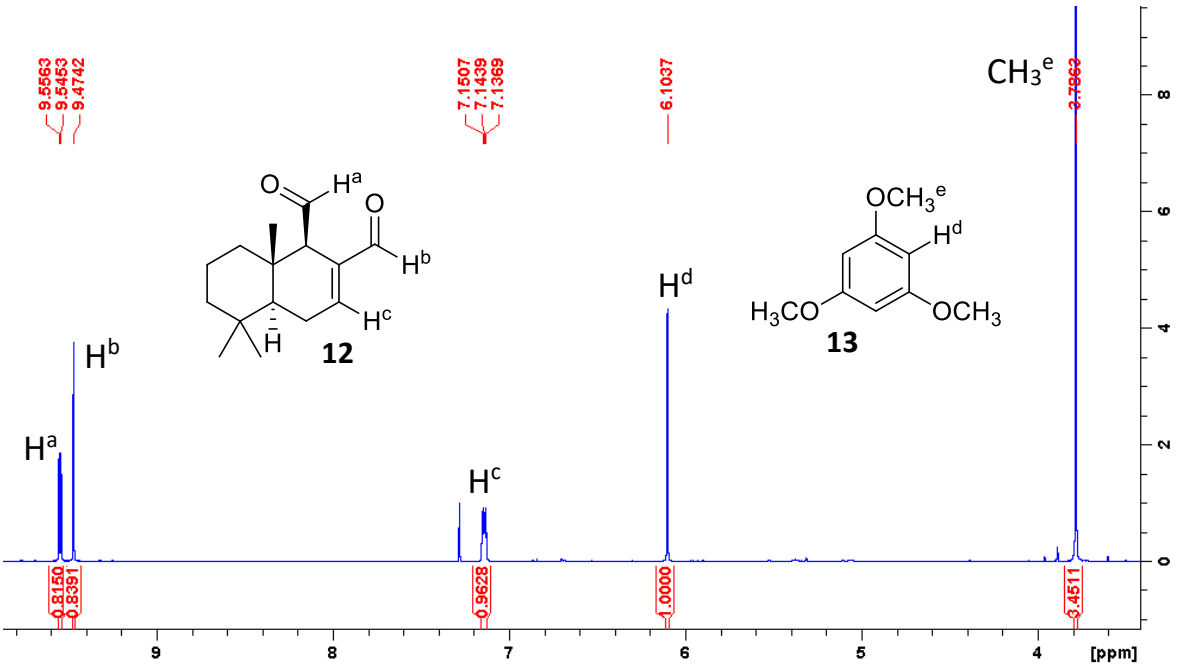


Figure 2.9 – ^{1}H NMR spectrum of polygodial with 1,3,5-trimethoxybenzene (~4:1 **12**:**13** by mass). Recorded using default parameters.

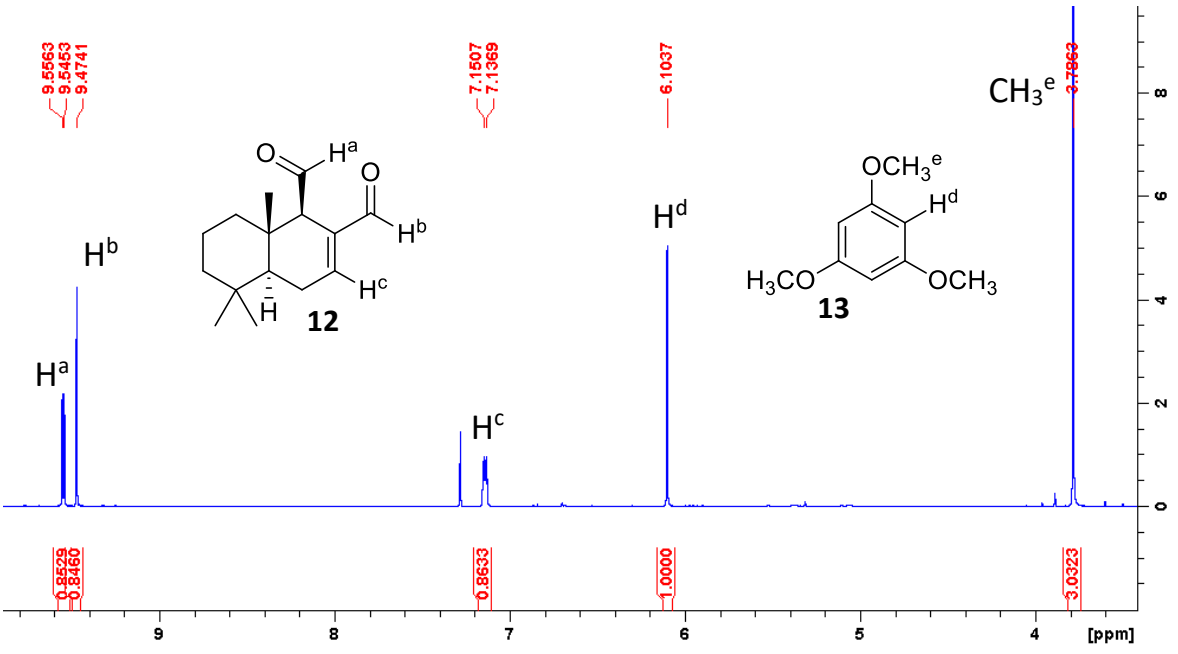


Figure 2.10 – ^{1}H NMR spectrum of polygodial with 1,3,5-trimethoxybenzene (~4:1 **12**:**13** by mass). Recorded using default parameters with the pulse width reduced to 30 °, and the relaxation delay increased to 10 s.

The results from the ASE optimisation study are reported in Figure 2.11 and Figure 2.12. It was observed that the highest percentage yield of polygodial was obtained at 90 °C and 35 % EtOH, which is fortuitously the closest matching conditions to the espresso

machine. At temperatures above 90 °C, significant epimerisation of the aldehyde was observed, leading to a mixture of polygodial and 9-epipolygodial (**14**). Decomposition was also observed at higher temperatures, with the ^{1}H NMR spectral data indicating more impurities. It was also observed that around the optimal conditions, the solvent composition has more influence on the extraction efficiency than the temperature. The highest yield recorded was 0.44 % w/w, which is significantly lower than the 0.7 % w/w achieved using the espresso machine PHWE method. The high temperature run with 100 % H_2O as the extraction solvent gave the lowest extraction yield of all the trials, and the high temperature caused significant decomposition.

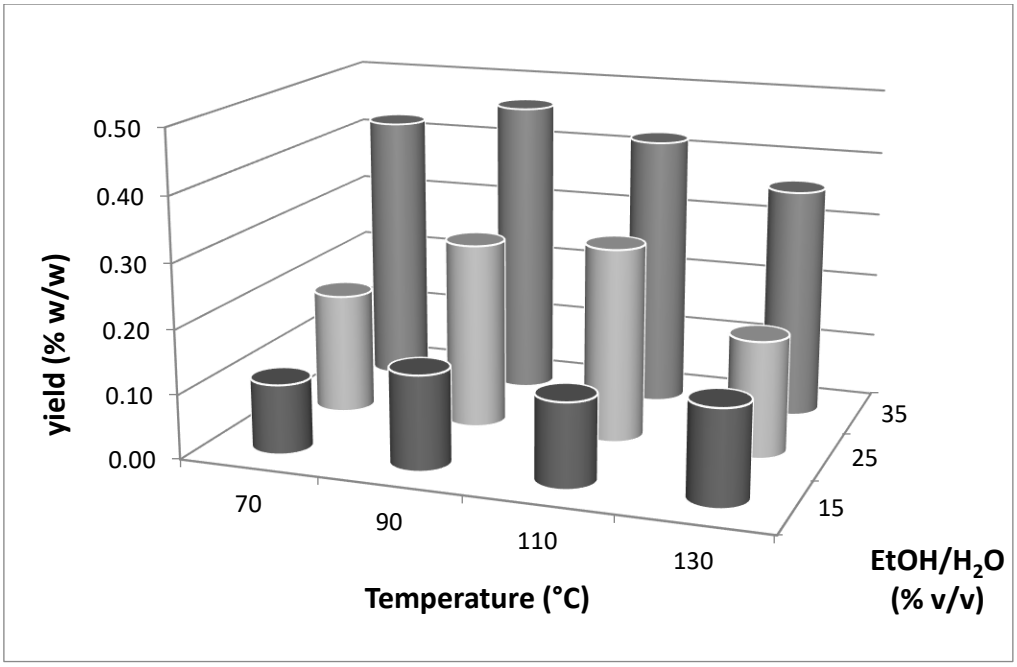


Figure 2.11 – Results obtained from the ASE200 PHWE optimisation study showing the yield of polygodial as a % (w/w) of the plant material extracted at 500 psi.

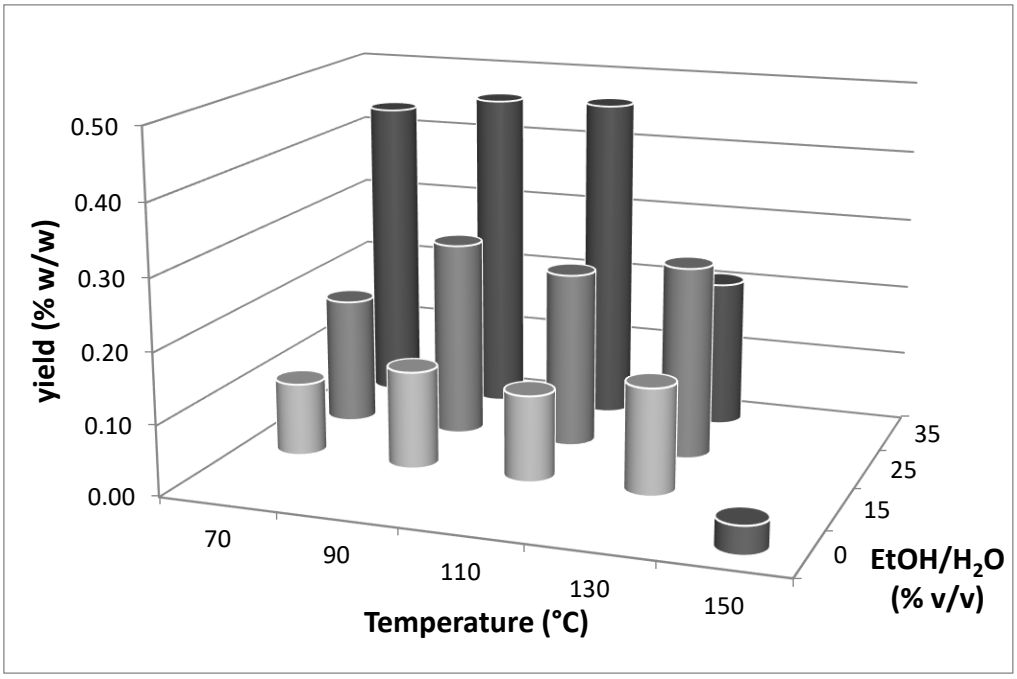


Figure 2.12 – Results obtained from the ASE200 PHWE optimisation study showing the yield of polygodial as a % (w/w) of the plant material extracted at 1500 psi.

One of the previously mentioned contributing factors to a loss of polygodial yield at higher temperature was the epimerisation of polygodial. The appearance of 9-epipolygodial was measured through the integration of the corresponding aldehyde peaks in the ^{1}H NMR at 9.88 and 9.43 ppm, which are completely separate from the polygodial aldehyde peaks at 9.48 and 9.56 ppm (Figure 2.14).[76,105] The epimerisation was calculated based on the ratio of polygodial to 9-epipolygodial observed, and shown in Figure 2.13 as a function of temperature.

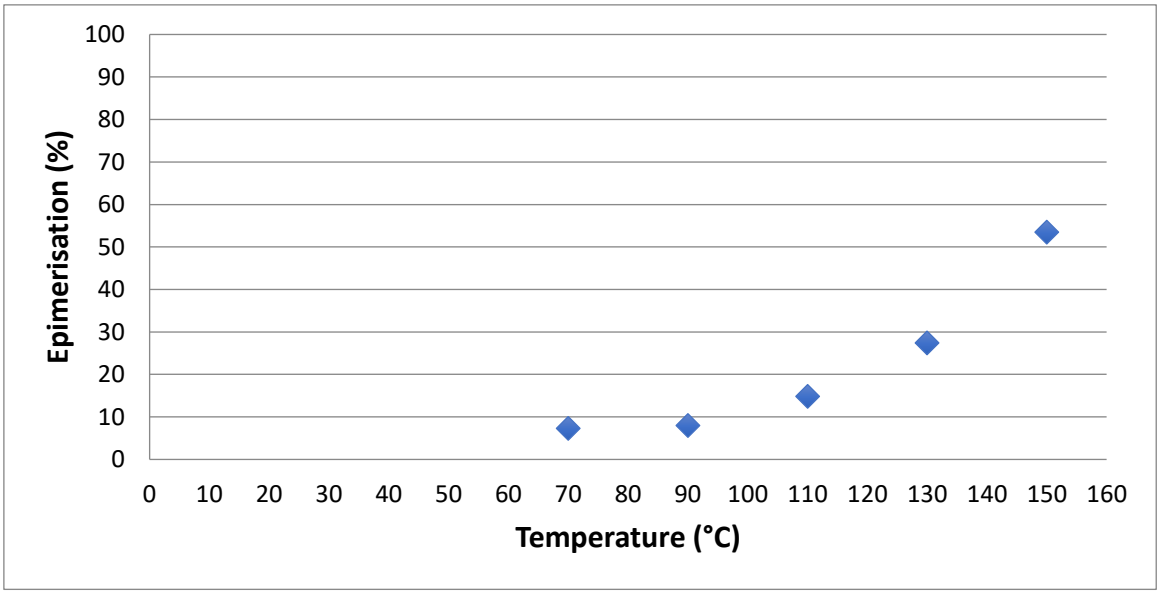


Figure 2.13 – Epimerisation of polygodial during extraction with the Dionex ASE200 as a function of temperature.

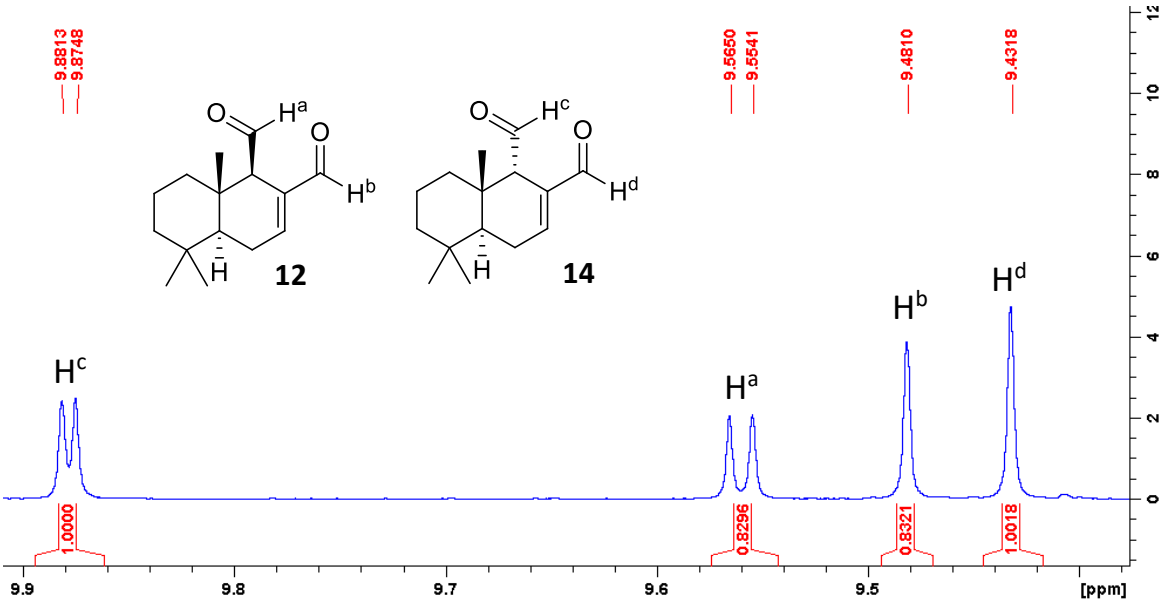


Figure 2.14 – Partial ^{1}H NMR spectrum showing polygodial and 9-epipolygodial in a mixture following epimerisation at 150 °C.

This epimerisation at 90 °C in the ASE extractions is at a much higher level than that observed for the espresso machine PHWE extraction, which is due to the much longer time at the extraction temperature (>20 mins vs ~2 min). The espresso machine extractions at 90 °C show negligible epimerisation, compared with nearly 10 % observed during ASE extraction. Even with epimerisation taken into account, the overall yield of

the extraction observed was highest at 90 °C and 35 % EtOH:H_2O v/v, and represents a lower yield than PHWE extraction of the same plant material as shown in Figure 2.15.

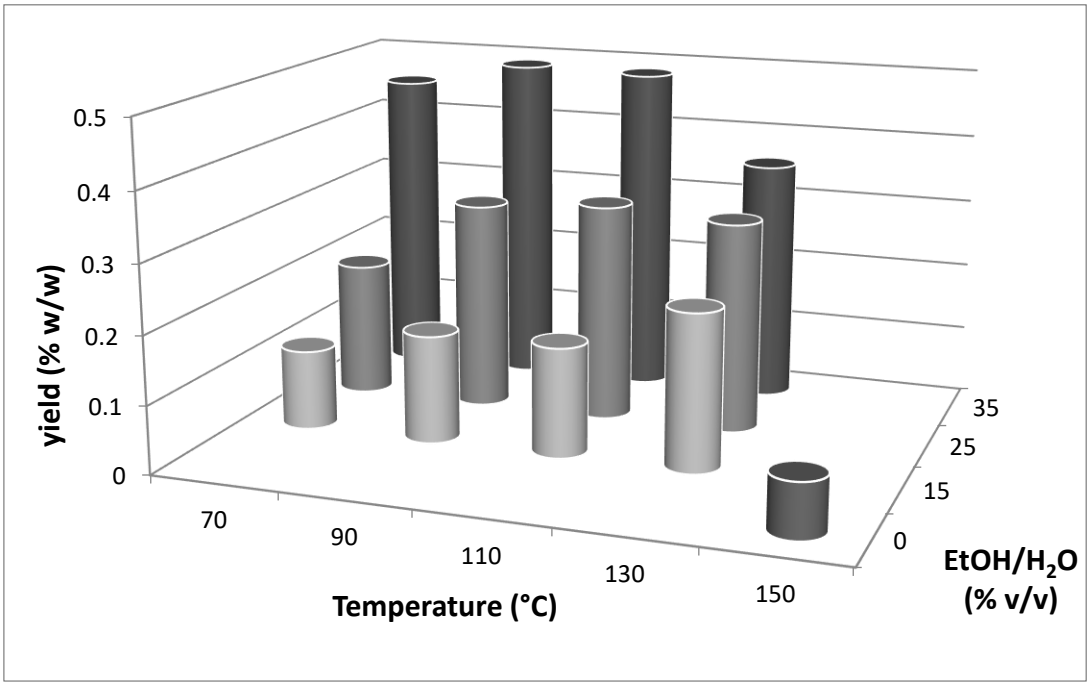


Figure 2.15 – Results obtained from the ASE200 PHWE optimisation study showing the combined yield of polygodial + 9-epipolygodial as a % (w/w) of the plant material extracted.

The minimal epimerisation observed in the espresso machine PHWE extracts is significant as it suggests that the method is not only efficient, but also that compounds with limited thermal stability may be routinely extracted. Further, due to the easy accessibility of the collection vessel in the espresso machine, the extract may be collected into a vessel containing ice to allow cooling immediately upon extraction, further improving the ability of the espresso machine to extract thermolabile compounds. This is not something which can be done with the ASE without modification of the instrument.

2.2.8 Isolation of Multi-Gram Scale Quantities of Polygodial, and Practical Considerations

Following optimisation of the PHWE extraction parameters for *T. lanceolata,* the method was scaled up to demonstrate speed and efficiency for the extraction of larger amounts of plant material. *T. lanceolata* ground leaf material was extracted (12 x 15 g), with the entire process taking around 30 minutes. From this PHWE extract, 6.5 g of crude material was obtained (3.6 % crude yield w/w) which was purified to yield 1.24 g of polygodial,

(0.7 % isolated yield w/w). The entire process was completed over an 8-hour period, which speaks to the rapid and efficient nature of the method.

Subsequently, samples of *T. lanceolata* were obtained that have specifically cultivated for their high polygodial content (variable, up to ~3.3 % w/w).* Applying the PHWE method to this material allowed the isolation of gram-scale quantities of polygodial efficiently within a matter of hours. The isolation of polygodial from this high yielding *T. lanceolata* crop was scaled up to the extraction of 120 g of leaf material, resulting in isolation of 2.44 g of polygodial after chromatography. This represents a yield of 2.0 % w/w, which is lower than the highest percentage observed of 3.3 % w/w. However, this was attributed to observed variation in the high yielding *T. lanceolata*, and still represents rapid isolation of gram-scale quantities of valuable material.

During these tests, after extraction with the espresso machine, the EtOH:H_2O extract was concentrated under reduced pressure to remove EtOH prior to secondary extraction. This additional step of evaporating the EtOH had two advantages. The resulting extraction volume was reduced which led to a simplified secondary extraction with dichloromethane, and the build-up of emulsions in the separating funnel was reduced.

Subsequently, ethyl acetate and heptane were trialled as less toxic alternatives for liquid-liquid extraction of the PHWE extract. All of these solvents were efficient in extraction of the polygodial from the aqueous PHWE extract, with the heptane showing the greatest selectivity, as assessed qualitatively by analysis of the ^{1}H NMR spectrum of the resulting extracts and by the colour of the resulting extract. A significant advantage of using heptane as the secondary extraction solvent is that during rotary evaporation, ~80–90% of the heptane can be readily recovered. Furthermore, when heptane was used as the extraction solvent, emulsions showed a much lower tendency to form during liquid-liquid extraction.

The reduction of volume of the extract was of more significance during scale-up experiments, as solvent extraction was limited to a volume of 1.5 L for practical and safety reasons. Evaporation of the EtOH became standard practice for an extraction when the secondary extraction solvent was CH_2Cl_2 or EtOAc, or when large volumes of

* Material provided by Essential Oils of Tasmania, www.eotasmania.com

extract were generated. Initially there were concerns that the polygodial may undergo thermal degradation or epimerisation by being held at a higher temperature for a longer time, as the water bath on the rotary evaporator required a temperature of 50 °C to allow efficient removal of solvent. However, no decreases in yield were observed, and the ^{1}H NMR spectrum of the extract obtained remained qualitatively unchanged following this process.

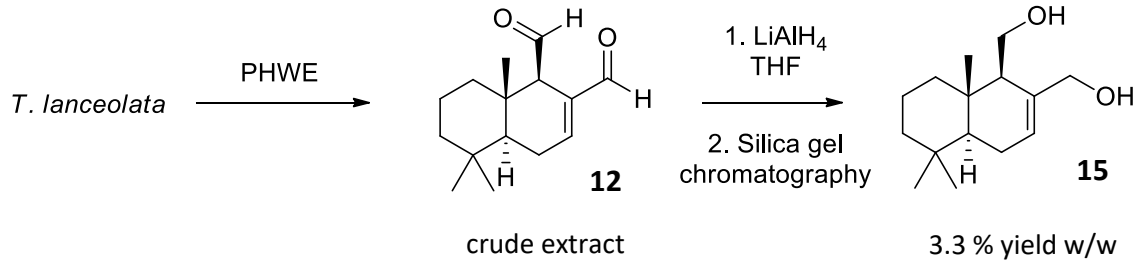
2.2.9 Multi-Gram Isolation of Polygodial by Crystallisation

It was subsequently discovered that from the high yielding crop of *T. lanceolata*, that polygodial could be isolated in gram-scale quantities rapidly without chromatography. The crude extract proved rich enough in polygodial when extracted with heptane that the compound could be readily obtained through crystallisation. When the material was crystallised without a chromatographic step, the crystals were typically of a small particle size and had an off–white/pale yellow appearance. In spite of the colour, the ^{1}H NMR spectroscopic analysis showed no evidence of impurities and the material was determined to be sufficiently pure for synthetic applications (Chapter 4). This type of process has a potential greater relevance as an industrial process, based on the proof-of-concept provided here. Pure white crystals were obtained if the material was subjected to silica flash column chromatography before crystallisation, with successive washing steps using ice-cold hexane. This resulted in a lower than optimal yield due to the solubility of the compound in hexane, but did provide polygodial of high purity in gram-scale quantities.

Figure 2.16 – Crystals of pure polygodial (**12**) obtained from chromatographed *T. lanceolata* heptane extract (left), off-white crystals of polygodial obtained through crystallisation of the crude heptane extract without a chromatographic separation (right).

2.2.10 Reactivity of Crude Polygodial Extract – Synthesis of Drimendiol

Though this method has shown that polygodial is able to be isolated rapidly with minimal degradation, many syntheses involving polygodial involve reduction of the aldehydes to furnish the known natural product drimendiol (**15**). Drimendiol is another natural product isolated from plants of the *Drimys* (Winteraceae) genus,[109] as well as some fungal species.[110] To this end, drimendiol may be isolated from *T. lanceolata* through reduction of purified polygodial, or by reduction on the crude extract prior to purification. Reaction on the crude extract may be a more efficient route to obtain this compound, as only one chromatographic step is required. To demonstrate this, the crude organic extract from *T. lanceolata* extraction was treated with $LiAlH_4$ in THF prior to purification. This process proceeded efficiently allowing expedited isolation of large quantities of drimendiol in up to 3.3 % yield (w/w).



Scheme 2.1 – Reduction of polygodial (**12**) with $LiAlH_4$ in the crude extract to form drimendiol (**15**).

^{1}H NMR spectroscopic analysis of the crude reaction product showed the loss of the aldehyde signals at 9.56 and 9.43 ppm, and the appearance of two sets of diastereotopic methylene signals at 4.30 ppm, 3.92 ppm, 3.85 ppm, and 3.61 ppm. This was indicative of the formation of hydroxymethyl groups in place of the aldehydes, indicating successful conversion to drimendiol. The material was purified by silica gel chromatography, resulting in drimendiol isolated in yields equivalent to the expected amount of polygodial in the extract.

2.2.11 Summary

Though PHWE would not typically be the first choice for the extraction of such a non-polar substrate, the method developed has shown high efficiency in extraction of

polygodial (**12**) from *T. lanceolata*. The rapid nature of the method has allowed the isolation of this sensitive substrate with limited epimerisation or decomposition. This was evident through the degree of enrichment of polygodial evident in the spectroscopic analysis of the crude extracts compared with extracts produced through ASE extraction or maceration. Further, the ease of purification of polygodial by flash column chromatography or crystallisation further highlights the degree of enrichment of the PHWE extract and limited extraction of plant pigments and therefore the viability of this method for extraction of non-polar terpenoids.

2.3 Shikimic acid from *Illicium verum*

2.3.1 Background

Shikimic acid and derivatives continue to be of interest in synthetic chemistry for a number of biological applications.[111-115] Indeed, in 2013 it was said of shikimate-derived natural products '…it is probably fair to say that terpenoid- and shikimate-derived natural products are now providing some of the most structurally interesting and biologically relevant target molecules of the 21st Century'.[116]

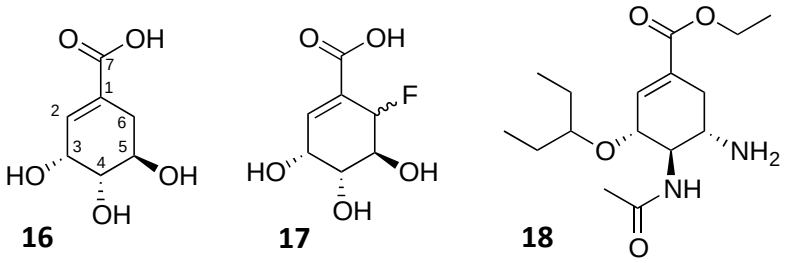


Figure 2.17 – Shikimic acid (**16**), fluorinated derivatives (**17**), and Oseltamivir (**18**) (the phosphate salt is the antiviral drug Tamiflu®)

Shikimic acid is a key intermediate in the shikimate pathway, used by plants and microbes to synthesize aromatic amino acids and other secondary metabolites.[117] Humans do not use this metabolic pathway,[118] but it is key to the life of plants, bacteria and also protozoan parasites. A key example is *Plasmodium falciparum*, the parasite that causes malaria.[119] Fluorinated shikimate derivatives (**17**) have been shown to be inhibitory to *P. falciparum* and have been tested as anti-malarial drugs.[119]

Shikimic acid derivatives also have other functions such as the anti-viral drug Tamiflu® (oseltamivir (**18**) phosphate) which acts as a viral neuraminidase inhibitor and is used to treat seasonal influenza, and avian and swine influenza outbreaks.[120] These properties make shikimic acid an attractive starting material for the synthesis of novel bioactive molecules. Further, due to the presence of the three contiguous stereocentres in the molecule, shikimic acid represents a versatile starting point in the asymmetric synthesis of synthetic targets.

In 2009, the influenza pandemic led to a greater than ten-fold surge in shikimic acid pricing, and methods to improve the acquisition of this compound were sought. Much of the shikimic acid was extracted from Chinese star anise grown in China, where the

extraction process was noted to be expensive, and yields dependent on weather conditions during growth.[121] At the time of this work being undertaken, the price of shikimic acid was relatively high. More recently, the price has decreased significantly due to industrial production of shikimic acid by microorganisms,[122] but is still around AUD$200 per gram.*

Shikimic acid is found in many plant species, but is noted to be in particularly high abundance in Chinese star anise (*Illicium verum* Hook. f.) fruit.[115] Dried star anise pods are inexpensive, and readily available in kilogram quantities. The quantity of shikimic acid in star anise varies, but reports estimate an average of 3.3 % by mass.[123,124] PHWE of shikimic acid has been previously undertaken, but on a custom built system for analysis of up to 0.5 g of sample at 150 °C at 150 bar pressure over 4 min.[125] Exhaustive yields were reported based on HPLC analysis, but the method was not practical for semi-preparative scale isolation. A method for larger scale isolation is therefore desirable.

With this in mind, investigations into the efficient extraction and isolation of shikimic acid and various protected intermediates were undertaken as a complementary method to other ways of generating this substrate. Through extraction of this plant, PHWE was further investigated to isolate significant quantities of the polar, carboxylic acid-containing compound shikimic acid from star anise. This represents an important expansion of the scope of the method, as initial method development was solely concerned with the extraction of polygodial, a C_{15} sesquiterpene.

2.3.2 Extraction and Chromatography Free Isolation of Shikimic Acid

For an initial test extraction, Chinese star anise was extracted with 30% v/v EtOH:H_2O, and subsequently extracted with dichloromethane. Shikimic acid is water soluble, and therefore secondary extraction with an organic solvent does not extract the shikimic acid. It does, however, remove the essential oil component from the extract to simplify the isolation of the shikimic acid. The essential oils which are present in yields over 8% w/w, and consist mainly of anethole.[126] The aqueous extract was subsequently evaporated, and analysis of the ^{1}H NMR spectrum of the crude extract in D_2O indicated that shikimic acid was the major organic component.

* As at 14/01/18 from Sigma Aldrich

Therefore, the extraction was repeated, silica gel and methanol were added, and the ensuing mixture was evaporated to dryness. The extract adsorbed onto silica was then loaded into a sintered glass funnel where different components of the extract could be successively eluted from the silica plug. The anethole and other essential oil components were removed by eluting with CH_2Cl_2 and EtOAc. Following this, the shikimic acid was eluted from the silica using 10% AcOH:EtOAc. This solvent system was of sufficient polarity to obtain the shikimic acid, while leaving behind many of the pigments and salts present in the extract. Upon evaporation of the solvent, an off-white solid was obtained which was further washed with a small quantity of CH_2Cl_2. ^{1}H and ^{13}C NMR spectroscopic analysis of the product obtained shows shikimic acid to be the major component, isolated in a 5.5% yield w/w in gram-scale quantities without chromatography.

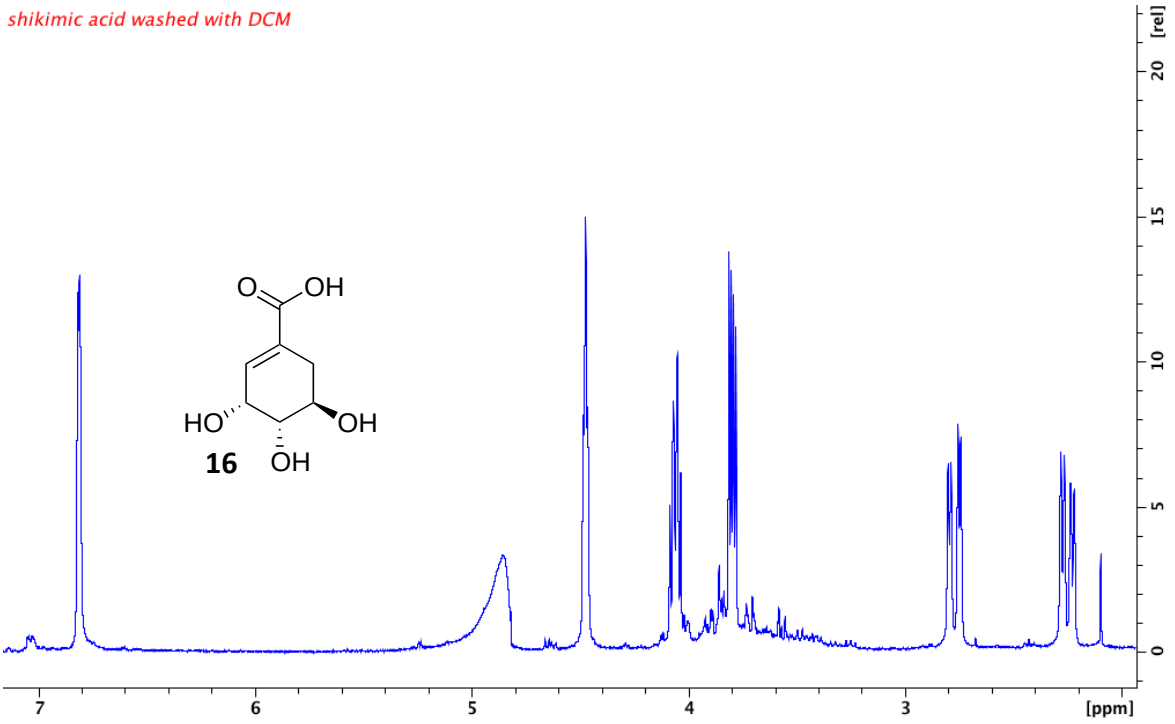
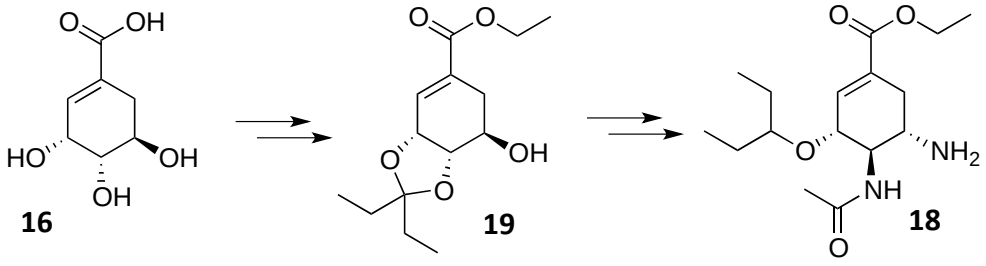


Figure 2.18 – ^{1}H NMR spectrum (D_2O) of shikimic acid isolated from *I. verum* without chromatography. (The unusual broad signal around 4.9 ppm represents a partially presaturated water signal.)

Figure 2.19 – Shikimic acid isolated from *I. verum* without chromatography.

2.3.3 Isolation of Protected Shikimic Acid Derivatives

While the isolation of shikimic acid directly provided material for synthesis, it was still impure (though this could be overcome with further purification by chromatography or recrystallisation). Additionally, synthetic work on this substrate often involves protection of the functional groups to form derivatives such as **19** and **20** as the first step.[127-130] To this end, protection reactions were directly carried out on *I. verum* crude extracts to isolate protected derivatives of shikimic acid.



Scheme 2.2 – Protected shikimic acid intermediate **19** in the synthesis of oseltamivir (**18**).

As many components of plant extracts are very polar such as salts, sugars and glycosides, the reduction in polarity of the desired substrate may allow for simplified purification. The reaction to convert shikimic acid to the methyl ester, and the *syn*-hydroxy substituents to an acetonide to form protected shikimic acid derivative **20** is reported many times in the literature.[130-134] A similar protected shikimic acid derivative (**21**) has been reported as an intermediate in the synthesis of marine natural product (–)-pericosine E (**22**).[135]

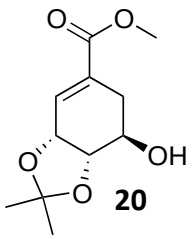
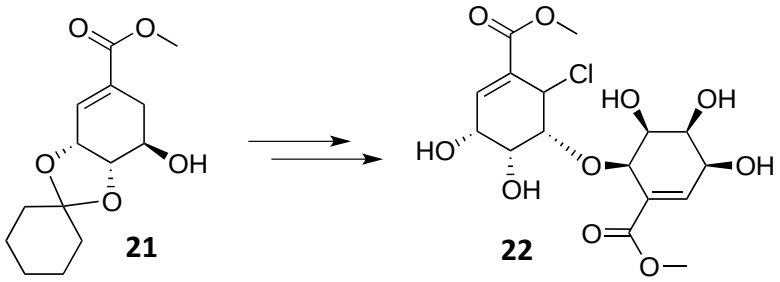
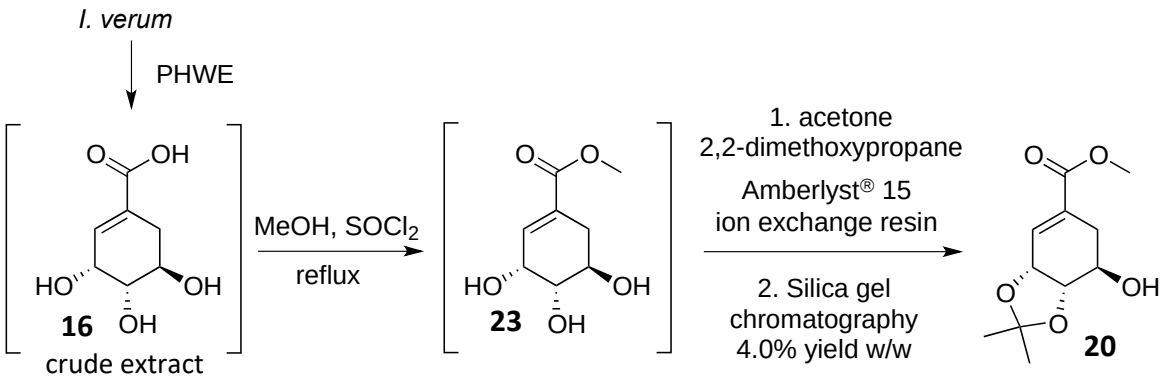


Figure 2.20 – Acetonide derivative of methyl shikimate



Scheme 2.3 – Protected shikimic acid derivative in the synthesis of (–)-pericosine E (**22**).

To test the protection of shikimic acid in the crude extract, 90 g of the ground pods were extracted by PHWE in 6 portions of 15 g. The extract was reduced in volume before washing with CH_2Cl_2. The residual aqueous fraction was then evaporated to dryness, and the resulting crude dark material esterified with HCl in MeOH to form methyl shikimate **23**. After evaporation of the MeOH, this material was then dissolved in acetone and 2,2-dimethoxypropane with Amberlyst® 15 acidic ion exchange resin added for the *syn*-diol protection of shikimic acid. Filtration and evaporation gave a black residue which was fractionated through a large silica plug, and subsequently purified by automated gradient flash chromatography to yield 4.76 g of protected shikimic acid derivative **20**, representing a 4.0% w/w isolation of shikimic acid. Notably, the entire process (from the extraction of star anise, to the purified compound) was undertaken within 8 h.



Scheme 2.4 – Reaction sequence to form methyl protected shikimic acid derivative **20**. Yield quoted represents equivalent weight of shikimic acid following extraction and protection followed by a single chromatographic step.

This 2-step protection protocol was then scaled up, to target ethyl derivative **19**, which is an intermediate in one of the syntheses of Tamiflu (Scheme 2.2, above).[136] In this case, extraction of 200 g of star anise seed pods was undertaken (10 x 20 g). Following concentration by rotary evaporation, and washing with CH_2Cl_2, the crude aqueous extract was then evaporated to dryness, and esterified analogously as described before, instead using EtOH to form ethyl ester **24**. Subsequent reaction with 3-pentanone and Amberlyst 15 acidic ion exchange resin provided the crude material. Purification via silica plug yielded 13.15 g of a yellow/brown oil of sufficient purity as determined by ^{1}H NMR spectroscopy, although an analytical sample could be provided by silica flash column chromatography. This mass represented a yield of 4.2 % w/w, and therefore a viable method to obtain protected shikimic acid derivatives for synthesis from an inexpensive source.

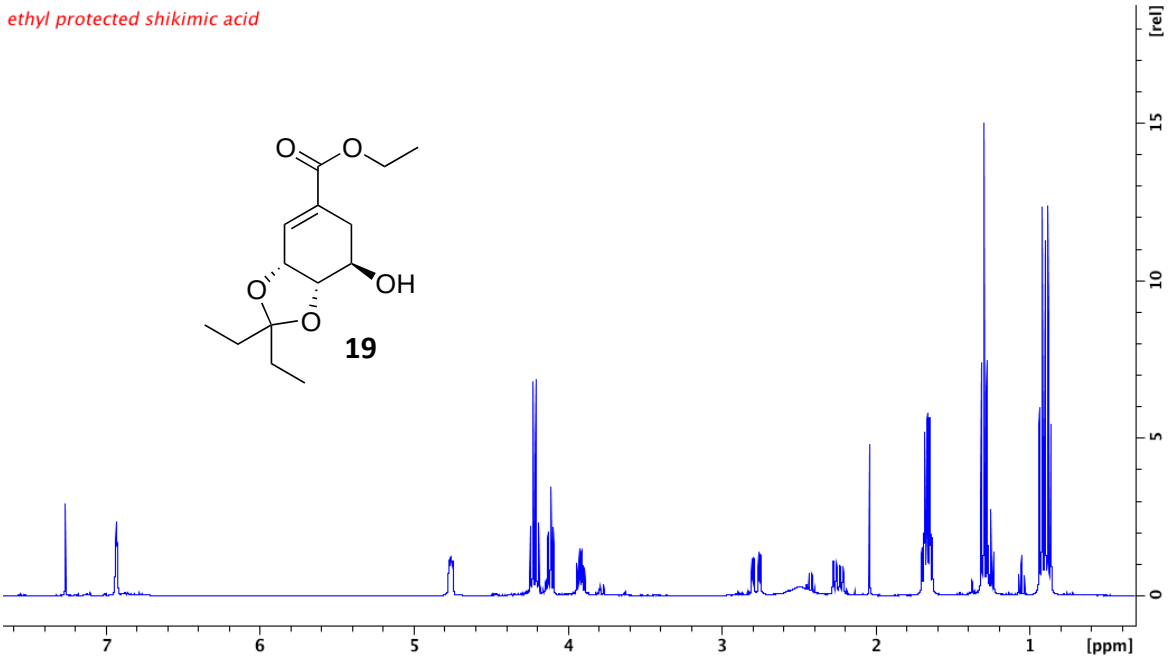
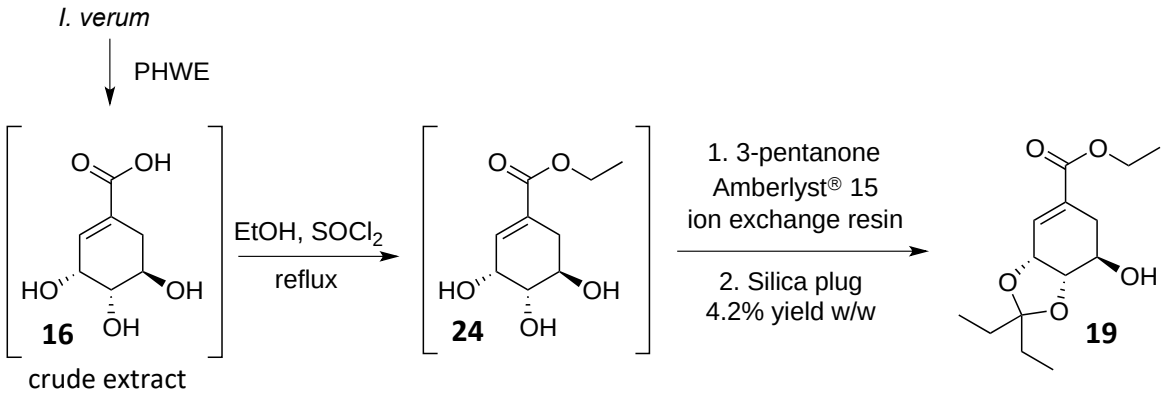
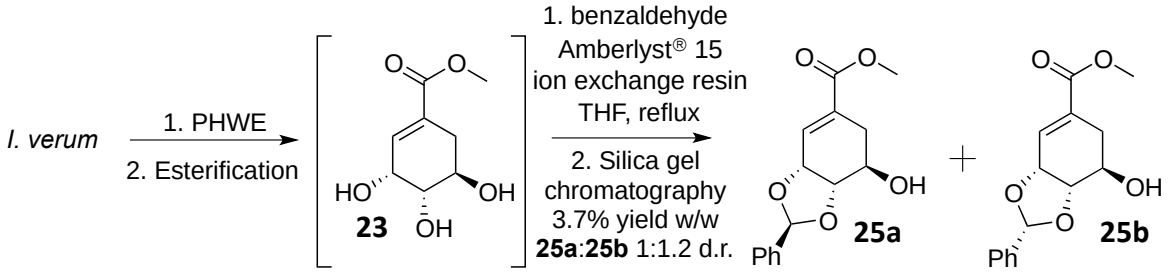


Figure 2.21 – ^{1}H NMR spectrum of the ethyl-protected shikimic acid derivative **19**.



Scheme 2.5 – Reaction sequence to form ethyl protected shikimic acid derivative **19**. Yield quoted represents equivalent weight of shikimic acid following extraction and protection followed by a single chromatographic step.

Following the successful isolation of gram-scale quantities of material from these two model studies, protection of the *syn*-diol with benzaldehyde as previously reported by Payne and co-workers[131] was undertaken. This yielded 3.7% (w/w) of the desired product as a mixture of diastereomers **25a** and **25b** (1:1.2 d.r.). Analysis of the ^{1}H NMR spectrum for this material shows clearly the formation of two diastereomers, with the key signals being the pair of singlets at 5.97 and 5.94 ppm representing the methine proton of the acetal adjacent the aromatic ring.



Scheme 2.6 – Synthesis of acetal diastereomers **25a** and **25b** with benzaldehyde. Yield quoted represents equivalent weight of shikimic acid following extraction and protection followed by a single chromatographic step.

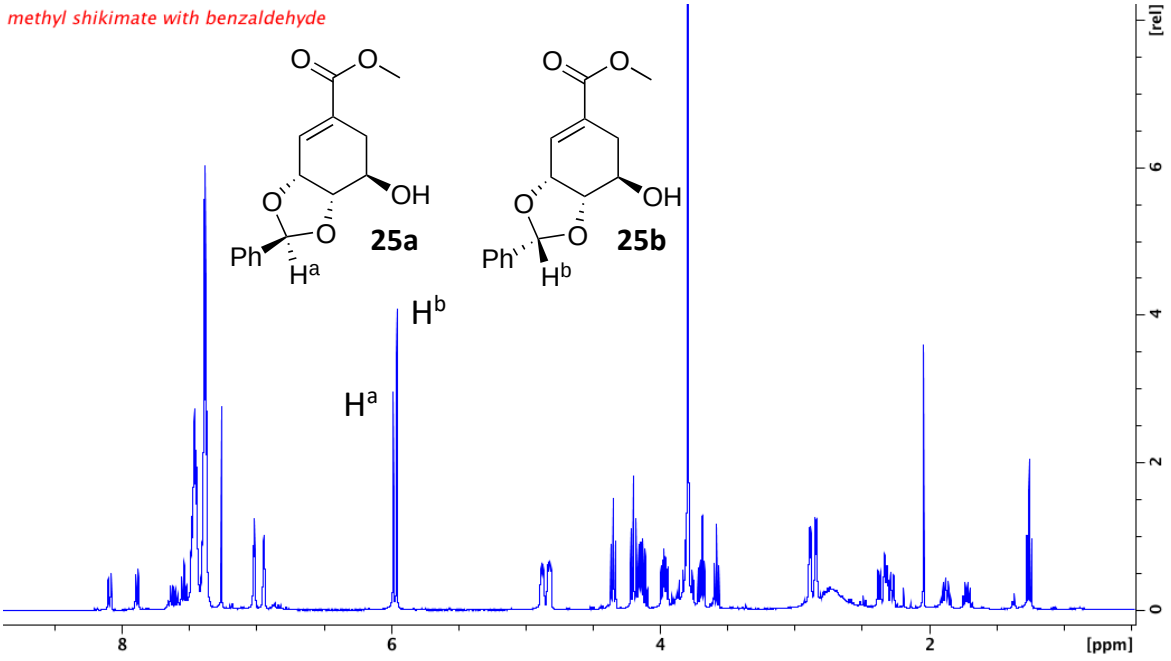


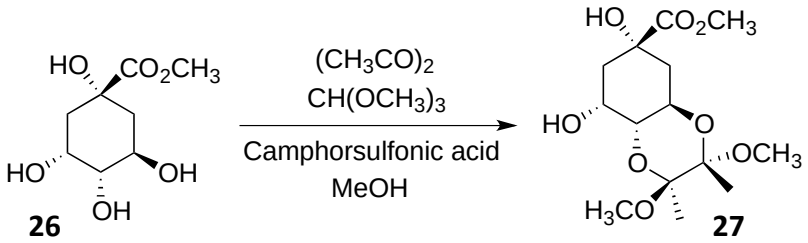
Figure 2.22 – ^{1}H NMR spectrum of the mixture of diastereomers **25a** + **25b**

2.3.4 Unsuccessful Protection Reactions

Protection of the hydroxy substituents at the 3- and 5- positions was attempted, which would leave a free hydroxy at the 4- position. This was based on a reported protection using TBDMS chloride which was shown to selectively protect these groups when only 2 equivalents of TBDMS chloride are added,[130] presumably due to steric influence. The problem with applying this method to the crude *I. verum* extract is that the amount of starting material, and the amount of other material in the crude mixture that could react with TBDMS chloride, is unknown. Excess TBDMS chloride could not be used however

due to the possibility of converting all three hydroxy substituents to the corresponding silyl ether. Unsurprisingly, this reaction afforded a mixture of unidentified products in negligible yield.

Protection of the *anti*-diol is also possible as reported by Armesto[137] using 2,3-butanedione as a protecting reagent in methanol under acidic conditions to form shikimate derivative **27**. The reported method uses camphorsulfonic acid in a sealed tube to go above the boiling point of methanol. A test was undertaken to determine whether this method could be made more facile by using Amberlyst® 15 resin and simple reflux in methanol, but no product was observed.

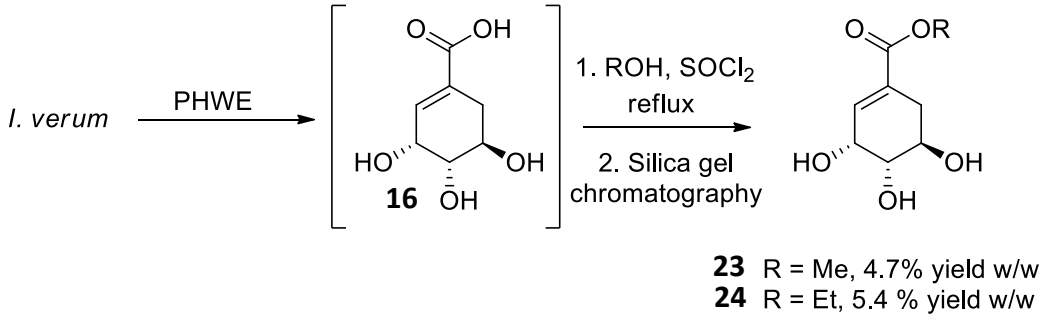


Scheme 2.7 – Protection of the *anti*-diol of a shikimate derivative reported by Armesto.

These more selective alcohol protection reactions are more sensitive to the conditions used, so it would be more practical to isolate esters **23** and **24** prior to protection of the hydroxy substituents.

2.3.5 Isolation of Shikimic Acid Esters

The methyl and ethyl esters were prepared analogously to above, and were found to be readily isolable. The methyl and ethyl ester were isolated successfully by automated gradient flash column chromatography eluting with MeOH:EtOAc in 4.7 % w/w (2.03 g from 40 g of Chinese star anise) and 5.4 % w/w (2.49 g from 40 g of Chinese star anise) yield respectively.



Scheme 2.8 – Esterification of the crude extract of shikimic acid to form esters **23** and **24**. Yields quoted represents equivalent weight of shikimic acid following extraction and protection, and a single chromatographic step.

2.3.6 Summary

Extraction of shikimic acid (**16**) from *I. verum* highlighted the viability of the method for the extraction of more polar compounds. In the same way as for for *T. lanceolata*, the enrichment of the extracts obtained by PHWE allowed for simple purification. Shikimic acid was isolated in gram-scale quantities without chromatography via fractionation by ion-exchange resin, Further, derivatisation of the shikimic acid to form less-polar substrates was efficiently achieved.

2.4 Coumarins from *Correa* Species

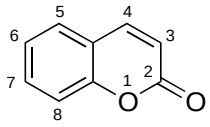
2.4.1 Background

As a result of a bioprospecting survey completed by the research group,[*] rapid and efficient isolation of milligram- to gram-scale quantities of coumarins from three species of *Correa* (Rutaceae) which are endemic to Australia – *Correa* reflexa ((Labill.) Vent.), *C. alba* (Andrews), and *C. backhouseana* (Hook.) was achieved. The aim of the survey was to investigate native or endemic Tasmanian plants, which had not been previously investigated, for natural product scaffolds. In this survey, each plant was extracted using the in-house developed PHWE method and there were two criteria that had to be satisfied in order for further investigation to be undertaken on the given plant. Given that the aim of the survey was to identify plant sources of organic scaffolds for potential synthesis applications, the criteria for further study for a given plant were major components identifiable in the ^{1}H NMR spectrum and TLC analysis of the crude extract,

[*] Bianca Deans, Honours Thesis, University of Tasmania 2014

and sufficient crude yield (≥1% w/w from 15 g of dried plant) following PHWE with 35% EtOH:H_2O, and subsequent liquid-liquid extraction with CH_2Cl_2. The chemistry of these *Correa* species has not been previously reported.[46] Following from this initial result from the survey, optimisation of the extraction yield was undertaken.

Coumarin compounds (2*H*-chromen-2-ones) are an important class of naturally occurring biologically active molecules extracted from a variety of plant species.[138-142] Owing to the diverse range of observed biological activities, coumarins are an attractive target for isolation from plant species, as well as being useful starting materials for synthesis of analogues.



28

Figure 2.23 – The simplest coumarin compound, coumarin (**28**, numbered).

C. reflexa was found to contain significant amounts of seselin (**29**) and (+)-epoxysuberosin (**30**). *C. alba* and *C. backhouseana* were both found to contain significant quantities of the coumarins alosin (**31**) and (–)-meranzin (**32**). *C. backhouseana* was also found to contain heraclenin (**33**). The similarity between C. *alba* and C. *backhouseana* compared with C. *reflexa* is consistent with the morphological similarity of the two, and also their relationship genetically, with *C. alba* and *C. backhouseana* being more closely related to each other than they are to C. *reflexa*.[143] All isolated coumarins in this study have an oxygen atom connected at the 7-position, and are further substituted at the 6- and/or 8- position. The isolated compounds are all known, and have previously reported biological activity**.**

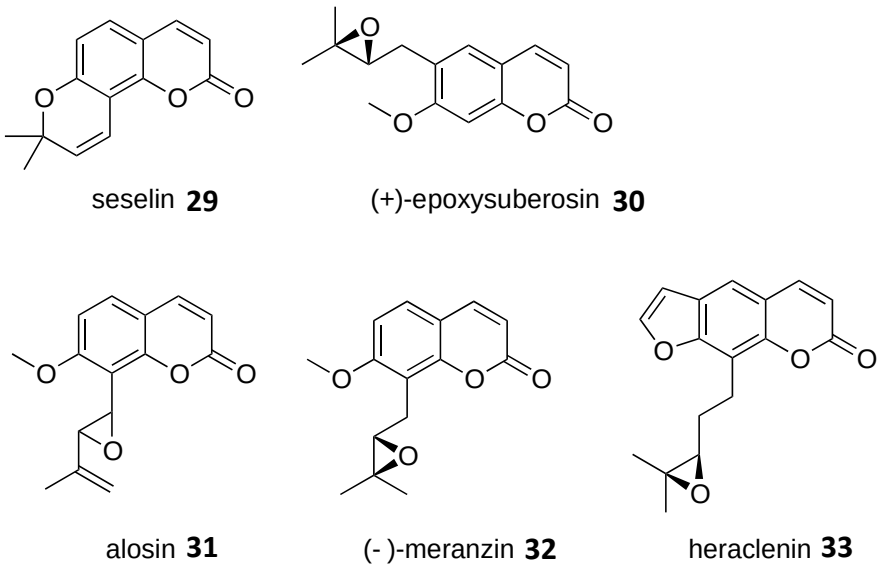


Figure 2.24 – Compounds isolated from *Correa* spp.

Seselin (**29**) has been shown to disrupt the development of blowfly larvae,[140] as well as showing antifungal[144,145] and anti-inflammatory activity.[146] (+)-Epoxysuberosin (**30**) has shown mild anti-tumor activity,[147] as well as being investigated for antioxidant activity, a trait many coumarins are known to possess.[148] Alosin (**31**) has shown antifungal,[149] anti-leishmanial[150] activity, as well as anti-proliferative activity towards human cancer cell lines.[151] The majority of reports of the bioactivity of (–)-meranzin (**32**) are concerned with that of the compound (–)-meranzin hydrate, in which the epoxide functionality has been ring-opened with water. Such activity includes anti-depressive[152] and anti-proliferative towards human prostate cancer cells.[153] This compound exists as a natural product[154] but may also be an artefact of isolation. Heraclenin (**33**) has shown phytotoxic,[141] antiplasmodial,[155] anti-bacterial,[156] nematocidal,[157] mutagenic,[158] and anticoagulant[159] activity. In addition, heraclenin has been studied as a potentially useful material in organic electronics due to its optical properties.[160]

Most of these compounds are available commercially, but their use in synthesis and biological applications is inhibited by a price range of $60–180 per mg.[46] Following the survey and identification of the major components, the method was further developed for this specific application and the yields of the compounds isolated increased significantly upon optimization. The main focus of the extraction of these compounds was to maximise the yield obtained from the extraction.

2.4.2 Liquid-liquid Extraction Solvent Optimisation for *C. reflexa*

C. reflexa was extracted by PHWE (35% EtOH:H_2O), and various parameters were investigated similar to the optimisation of the extraction of *T. lanceolata* described previously. It was determined for the *Correa* spp., that the maximum amount of plant material that would fit into the sample compartment of the espresso machine was 10 g, in comparison to 15 g for the *T. lanceolata*, and 20 g for *I. verum*. This was simply due to the physical nature of the leaves.

An extraction of *C. reflexa* (10 g) was undertaken, and the resulting PHWE extract then extracted successively with CH_2Cl_2 and EtOAc. Both organic extracts contained 2 major identifiable components, seselin (**29**) and (+)-epoxysuberosin (**30**). The combined yield of 366 mg represented a yield of 3.7 % w/w from dry plant material, compared with 2.6 % w/w when using CH_2Cl_2 extraction alone. As these extracts contained the same components, this suggested that CH_2Cl_2 was not an appropriate extraction solvent for the efficient exhaustive extraction of the coumarin compounds from the PHWE extract. An important feature of the ^{1}H NMR spectra for *C. reflexa* is the lack of impurities. Indeed, for this extract, a single chromatographic separation was required to isolate the two major components.

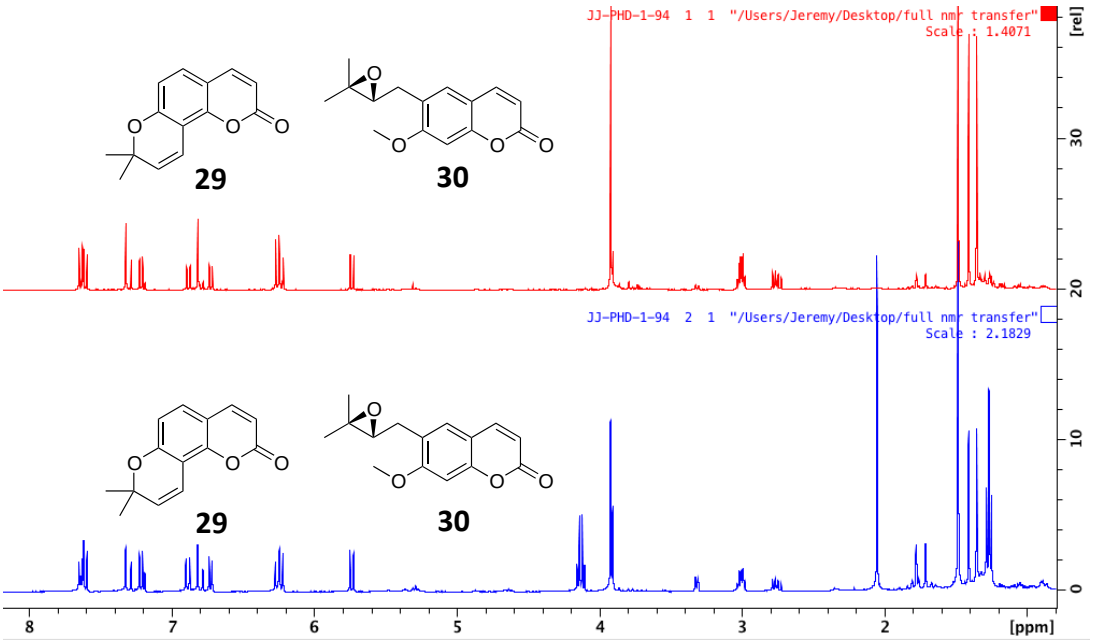


Figure 2.25 – ^{1}H NMR spectrum of the CH_2Cl_2 extract (above) and the subsequent EtOAc extract (below) of *C. reflexa* (contains residual EtOAc)

2.4.3 PHWE Solvent Volume Optimisation for *C. reflexa*

A sample of *C. reflexa* was then extracted by PHWE (3 x 100 mL, 35% EtOH:H_2O) to determine if the PHWE extraction is exhaustive for the compounds. Each fraction was then extracted with EtOAc, and following removal of solvent, fraction 1 contained 266 mg, fraction 2 contained 103 mg, and fraction 3 contained 16 mg. A qualitative assessment of the ^{1}H NMR spectrum of these extracts also determined that the last fraction was the least enriched in the coumarin compounds. This was consistent with the result observed for *T. lanceolata*, showing that 200 mL of extraction solvent from the espresso machine is sufficient for extraction of the desired material from the plant, and that a higher volume increases the levels of undesired components being extracted.

2.4.4 Scaled-Up Extraction of *Correa* Species

Following the optimisation of key parameters, it was determined that EtOAc should be used for liquid-liquid extraction of the PHWE extract, instead of CH_2Cl_2. Subsequently, large-scale extractions were carried out on all species, and the components isolated to purity. The results are summarised in Table 2.3, with comparison to the yields previously obtained by Deans.*

	C. reflexa		*C. alba*		*C. backhouseana*	
	% of dry plant weight	Mass isolated (g)	% of dry plant weight	Mass isolated (g)	% of dry plant weight	Mass isolated (g)
seselin **29**	1.00 (0.14)	1.00	-	-	-	-
(+)-epoxysuberosin **30**	1.15 (0.25)	1.15	-	-	-	-
alosin **31**	-	-	0.89 (0.37)	.268	0.065 (0.079)	0.065
(–)-meranzin **32**	-	-	0.31 (0.19)	.094	0.050 (0.051)	0.050
heraclenin **33**	-	-	-	-	0.041 (0.029)	0.041

Table 2.3 – Compounds isolated from *Correa* spp. with % yield w/w. Numbers in parentheses represent the initial yields reported from the bioprospecting survey by Deans.* The primary difference in the method was use of EtOAc instead of CH_2Cl_2.

* Bianca Deans, Honours Thesis, University of Tasmania 2014

For *C. reflexa*, extraction of 100 g of material (10 x 10 g) was undertaken. After concentration by rotary evaporation, subsequent EtOAc extraction provided an extract in 3.9 % w/w yield. Purification by automated flash column chromatography yielded the compounds seselin (**29**) and (+)-epoxysuberosin (**30**) in 1.0 and 1.15 % yield (w/w) respectively. *C. alba* (3 x 10 g) was extracted, yielding alosin (**31**) in 0.89% yield w/w and (–)-meranzin (**32**) in 0.31 yield w/w. *C. backhouseana* (10 x 10 g) also contained the major components, alosin (**31**) (0.065 % yield w/w) and (–)-meranzin (**32**) (0.050 % yield w/w) as well as an additional component, heraclenin (**33**), isolated in 0.041% yield (w/w). For all of these extractions, no ring-opening of the epoxide moieties was observed, again demonstrating the mild nature of this extraction method.

These experiments show the difference in extraction yield, influenced by a combination of factors. Level of experience with the technique, optimisation of pressure based on flow rate, and secondary extraction solvent have all played a part in increasing the yield of the coumarin compounds isolated from these *Correa* species. The increase in the yield obtained increased up to ~7x greater in the case of seselin, isolated from *C. reflexa*. The above table summarises in full the compounds isolated from the scaled up and optimised extraction of the 3 *Correa* species. The isolation of compounds from *C. backhouseana* did not show an increase in yield, however, *C. reflexa* and *C. alba* gave a significant increase in yield following the optimisation.

2.4.5 Summary

The extraction of gram-scale quantities of valuable coumarins (**29**–**33**) from three *Correa* species further highlights the application of this method for the extraction of sensitive non-polar substrates. These compounds were potentially unstable to the extraction conditions due to their epoxide moieties. However, as for polygodial, the reaction or degradation of these substrates was not observed. The NMR spectroscopic analysis of the extracts of these plants showed a high concentration of the targeted coumarin compounds, resulting in simple purification of these compounds by flash column chromatography. These results support the use of the PHWE method for screening plants for natural products, as degradation of sensitive substrates is limited.

2.5 Conclusion

Through the rapid extraction of the three plants described, the viability of an unmodified espresso machine for PHWE has been conclusively demonstrated. The extract for *T. lanceolata* was demonstrated to be more enriched in the desired compound, and therefore more facile to purify compared with maceration extracts. For all the given compounds, Soxhlet extraction would require specialised glassware, and is noted to take a significantly longer time compared to this PHWE method. In addition, the isolated compounds would not be viably extracted through hydrodistillation due to their high boiling points. Modern techniques such as supercritical fluid extraction may prove to be efficient ways to extract some of these compounds, however, the apparatus is specialised and expensive and therefore would not be compatible with the aims of this project.

Though this method is not always going to be the optimal choice for all natural product extractions, it has proven to be faster and more cost effective than the methods described in the introductory chapter of this thesis for the examples given. This new method represents an efficient, rapid and cost-effective complementary technique for the isolation of gram-scale quantities of valuable and sensitive organic compounds.

Chapter 3: Applications of the PHWE Method

3.1 Overview

In this chapter, the PHWE method was further investigated though the extraction of various plant species ranging from invasive plants, commercial crops and common herbs. These species were chosen for a variety of reasons to demonstrate the broad applications of this new method.

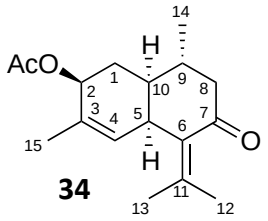
Thirteen different plant species were investigated to provide a broad range of examples, each increasing the understanding of the scope and utility of this method. *Eupatorium adenophorum, Chrysanthemum cinerariaefolium, and Momordia charantia* were extracted to rapidly generate extracts for biological applications. *Castanospermum australe* and *Marrubium vulgare* were extracted as they are known to contain valuable natural products that are used extensively for biological studies, or further synthetic studies. Extraction of *Drimys winteri*, and a survey of *Tasmannia lanceolata* were undertaken to further investigate the viability of the method as a rapid screening and bioprospecting tool. Finally, extraction of the essential oils from 6 common culinary plants were undertaken to investigate the potential of the method as an alternative to distillation, and as part of efforts to translate the method into an undergraduate laboratory natural products extraction technique.

3.2 Rapid Generation of Bioactive Extracts

3.2.1 Terpenes and Glycosides from Crofton Weed (*Eupatorium adenophorum*)

3.2.1.1 Background

Eupatorium adenophorum (Spreng.) (Crofton weed) is an invasive plant species, which contains several cadinane type sesquiterpenes. The basic structure of many of these compounds was reported in the late 70's[161] and early 80's.[162] However, subsequent analysis and X-ray studies on this class of molecules led to the revision of the absolute stereochemistry.[163,164]



34

Figure 3.1 – Representative example of cadinane sesquiterpene **34**, showing numbering system

In spite of the correction of the absolute stereochemical assignment of the cadinane backbone in *E. adenophorum*, the incorrect absolute stereochemistry is shown in more recent reports.[165,166] Further, in a number of instances the stereochemical information has been omitted entirely.[167,168] Historically, study of Crofton weed became important in part due to the toxicity of the plant to horses. Horses were noted to go into respiratory distress and develop lung damage if allowed to continually consume the plant, indicating that metabolites of this plant possessed interesting biological properties.[169,170] Additionally, Crofton weed extracts have been extensively studied for their allelopathic (seed germination and growth inhibition) effects against various plant species.[171-178]

The original report of the cadinane components, reported by Bohlmann and Gupta, describes the isolation of six cadinene terpenes. The structures are shown in Figure 3.2, below. The structures provided in the original paper are of the opposite absolute stereochemistry, which was revised after publication as described above. In this report, 320 g of plant material was extracted to yield 160 mg combined cadinanes, representing a total yield of ~0.05% w/w cadinanes.[162]

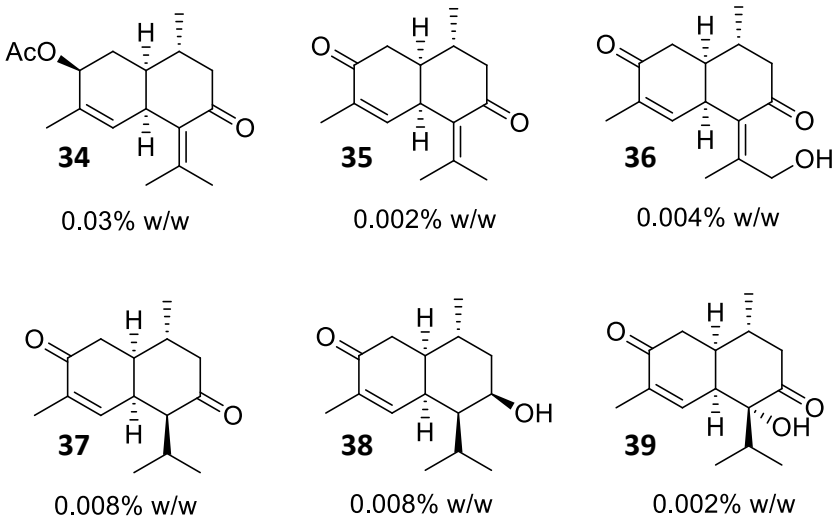


Figure 3.2 – Originally reported cadinanes by Bohlmann and Gupta. The original report showed the enantiomers, which were subsequently revised.

Many reports of the isolation of cadinane compounds form *E. adenophorum* have shown inefficient extraction and featured labour- and time-intensive procedures requiring large volumes of solvents, and multiple chromatographic procedures, yielding compounds in yields as low as 0.00002 % w/w. For example, He and co-workers report the extraction of 31 kg of *E. adenophorum* plant material using an unspecified volume of methanol. The resulting extract was subsequently fractionated by suspension in water, and extraction with petroleum ether, chloroform, and EtOAc. Multiple flash column chromatographic steps and recrystallization were required to isolate the cadinane components of this extract. In this way, four cadinane derivatives (509 mg total mass) were isolated (Figure 3.3), representing a yield of 0.0016% w/w.[179]

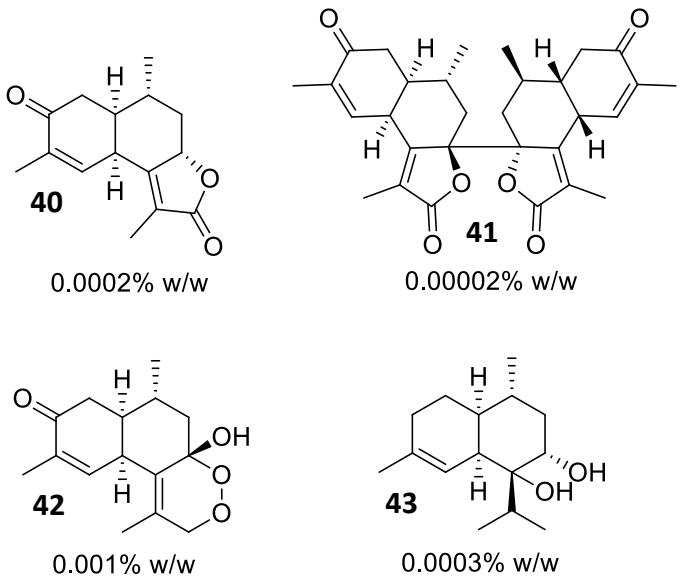


Figure 3.3 – Cadinane terpenes isolated by He and co-workers.

In another example, Li and co-workers report the extraction of 10 kg of *E. adenophorum* plant material, using 75 L of petroleum ether, followed by multiple column chromatography steps requiring litres of chloroform/petroleum ether mixtures to isolate five known and four previously unreported cadinane derivatives (Figure 3.4) in a yield of 0.0017% w/w (170 mg total mass).[172]

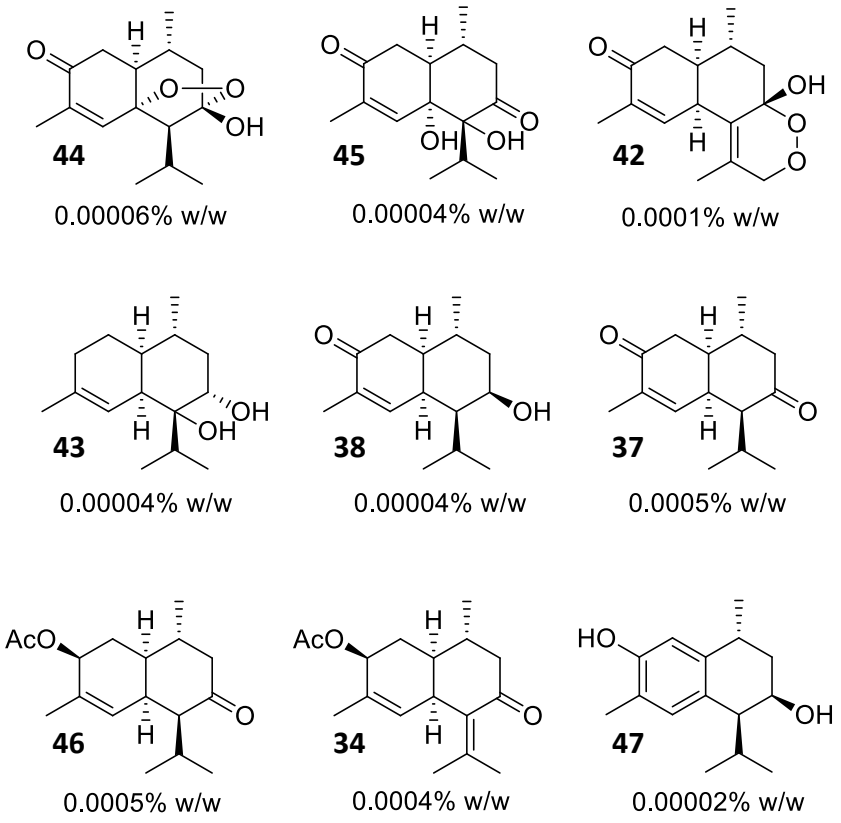


Figure 3.4 – Cadinane terpenes isolated by Li and co-workers.

Other examples of cadinanes isolated in low yields from this plant include Shi and co-workers report of the isolation of cadinane terpene **48**, in a 4 mg yield from 1 kg of leaves, after 4 flash column chromatographic steps. This represents a yield of 0.0004 % w/w.[180] Additionally, a separate report from He and co-workers describes the isolation of cadinane **49** from 1 kg of leaf material (21 mg, 0.002% yield w/w). The dried plant was extracted with methanol for 6 days, suspended in water, and fractionated into petroleum ether, chloroform, ethyl acetate and n-butanol. The chloroform fraction was subjected to multiple flash chromatographic steps and recrystallization to yield this compound.[181]

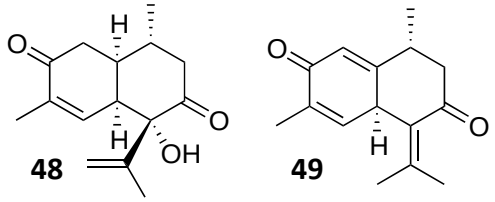


Figure 3.5 – Cadinane terpenes **48** and **49**.

A review of all compounds isolated from *E. adenophorum* was completed in 2015, containing all reports up to 2013, including those discussed above.[182] The revision of

stereochemistry for these compounds was not mentioned in the review, with the incorrect stereochemistry for these compounds reported therein.

Collaborators at the School of Biological Sciences at the University of Tasmania were interested in investigating the allelopathic effects previously discussed. Therefore, PHWE was investigated as a tool to extract compounds from this plant. Given the inconsistencies in previous reports, and the large number of compounds that have been reported, the aim of this extraction of this plant was to extract, isolate, and quantify where possible the major cadinane sesquiterpenoids. Subsequently, our collaborators used these compounds in their biological studies.

3.2.1.2 Extraction and Isolation of Heptane Soluble Components

The plant was extracted by PHWE with 35% EtOH:H_2O, and the resulting crude aqueous extract was extracted with heptane to yield a fraction composed primarily of terpene-type compounds. The ^{1}H NMR spectrum of the heptane extract is shown below In Figure 3.6. A number of major components are evident in the spectrum.

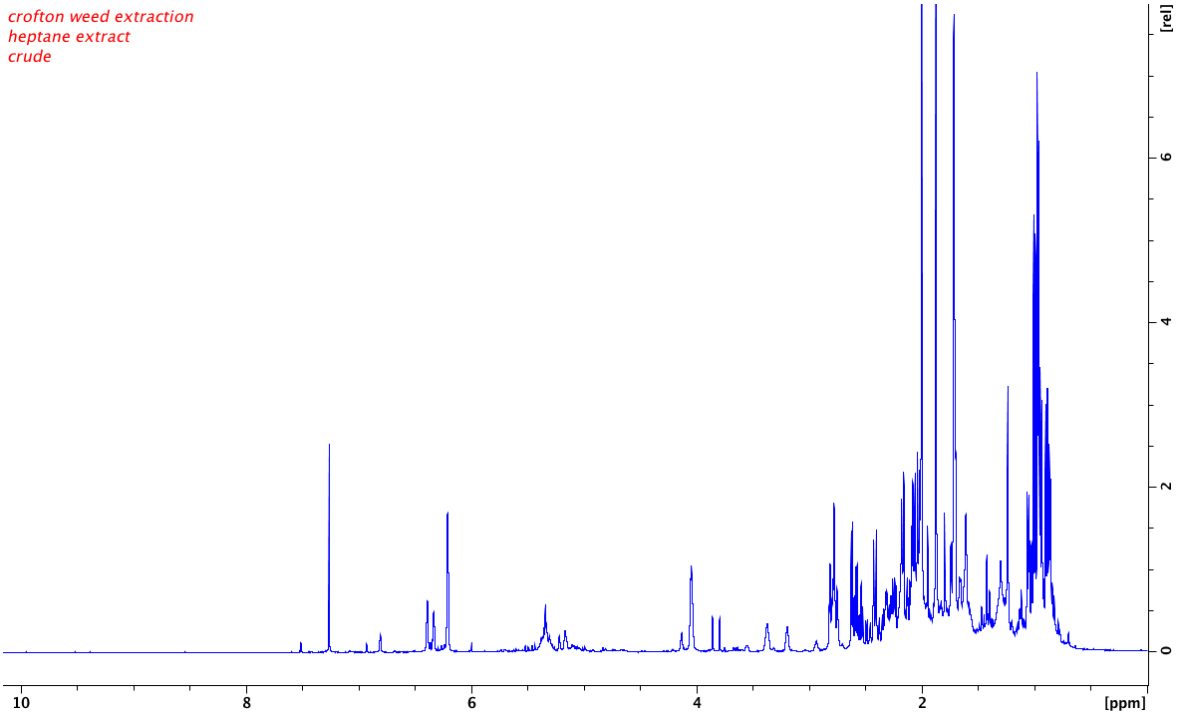


Figure 3.6 – ^{1}H NMR spectrum of the heptane extract of *E. adenophorum*

Purification of the heptane extract by automated flash gradient chromatography provided the chromatogram shown in Figure 3.7. The peaks between 8 and 20 minutes in

the separation profile were analysed, and it was found that some of these peaks contained multiple compounds. This was not an unexpected result, as there are many compounds previously reported within this plant which are very similar in structure and polarity. The peaks are labelled 1 through 7 for the purposes of discussion.

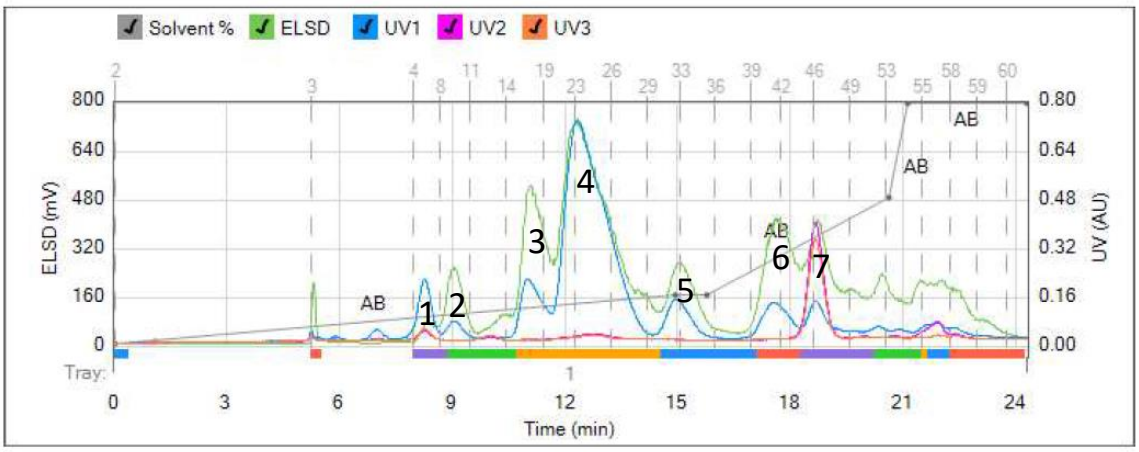


Figure 3.7 – Separation profile of the *E. adenophorum* heptane extract, major peaks are numbered 1 through 7.

As some of the peaks in the chromatogram contained multiple components, some compounds required multiple chromatographic steps to obtain pure material for characterisation. Other peaks contained pure fractions which could be directly identified without further chromatography. With the exception of compound **50**, all the compounds isolated and identified from this extract were cadinane type sesquiterpenoids. Of these compounds, seven were known and three were previously unreported. A summary of the compounds isolated from this heptane extract from *E. adenophorum* is provided in Figure 3.8 and Table 3.1, below.

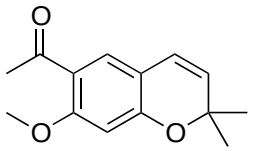
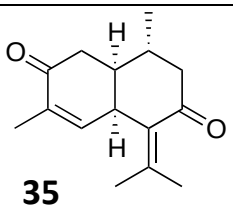
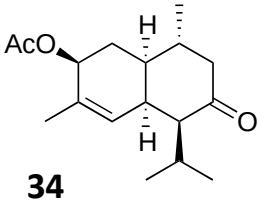
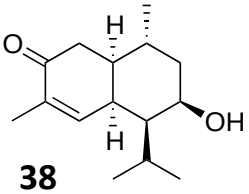
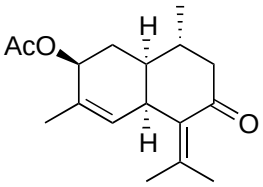
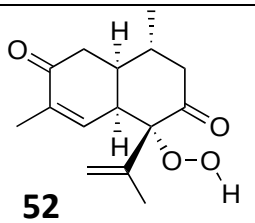
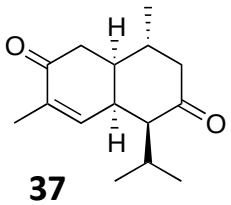
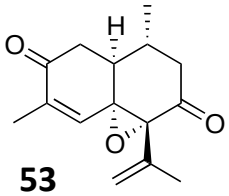
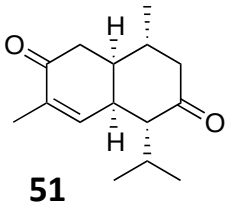
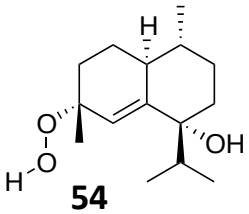
Structure	Yield (mass, % w/w)	Ref	Structure	Yield (mass, % w/w)	Ref
 **50**	4 mg, 0.02 %	183	 **35**	9 mg, 0.05 %	168
 **34**	5 mg, 0.03 %	165	 **38**	30 mg, 0.15 %	164
 **46**	3 mg, 0.02 %	168	 **52**	12 mg, 0.06 %	-
 **37**	63 mg, 0.32 %	164	 **53**	5 mg, 0.03 %	-
 **51**	56 mg, 0.28 %	164	 **54**	Crystal only isolated	-

Table 3.1 – Compounds isolated from the heptane extract of *E. adenophorum*

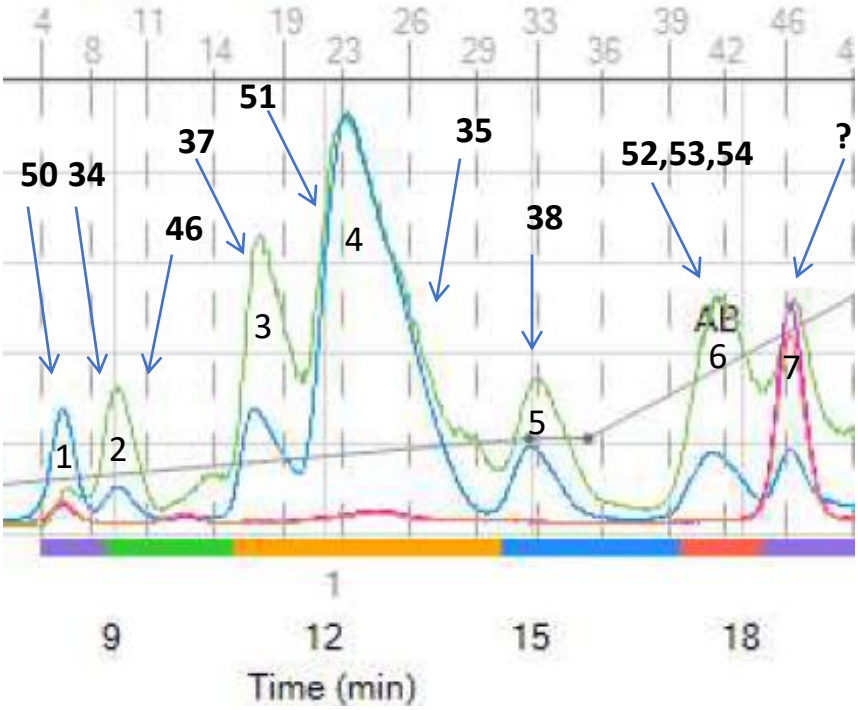


Figure 3.8 – Chromatogram showing the separation profile for the compounds isolated and identified from *E. adenophorum* heptane extract.

NMR spectroscopic analysis of peak 1 provided a spectrum that was not consistent with a cadinane sesquiterpene, and the compound was subsequently identified as compound **50** through comparison with literature data.[183] This compound has been previously reported as isolated from *E. adenophorum*,[184] and has been also been found in other plant species such as *Tithonia diversifolia*[183] (Mexican sunflower). Key features of the ^{1}H NMR spectrum differentiating this compound from the cadinane terpenes were the aromatic singlets at 7.53 and 6.38 ppm, and the singlet corresponding to the *gem*-dimethyl groups in the pyran ring at 1.44 ppm. Singlets integrating for 3 protons at 3.9 and 2.56 ppm were consistent with the methoxy and acetoxy functionalities of the molecule, and the alkene signals at 6.30 and 5.52 ppm had a coupling constant of 9.9 Hz, consistent with a *cis*-configuration.

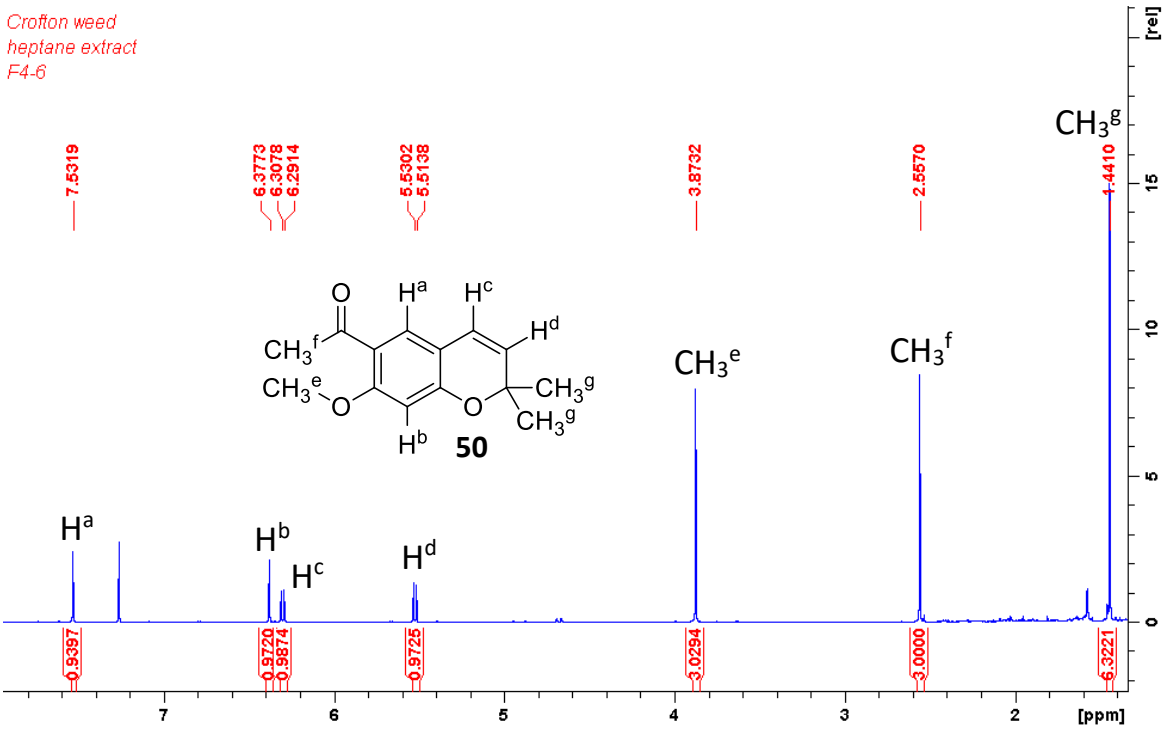


Figure 3.9 – ^{1}H NMR spectrum of compound **50**.

Peak 2 required a second flash chromatographic step to isolate known compounds **34**[165] and **46**.[168] The difference between these two compounds was the nature of the bond between C-6 and C-11. In compound **34** this was a single bond, and in compound **46** this was a double bond. The key differentiating signals between these two compounds were the signals for the methyl groups at the C-12 and C-13 positions. For compound **34**, these signals were doublets around 1 ppm, which were split by the adjacent methylene proton attached to C-11. In compound **46**, these signals were singlets closer to 2 ppm, which is consistent with attachment to an sp^2 hybridised carbon atom of an alkene. The shift of the methine proton signal from 2.72 ppm in compound **34** to 3.57 ppm in compound **46** supports the position of the alkene.

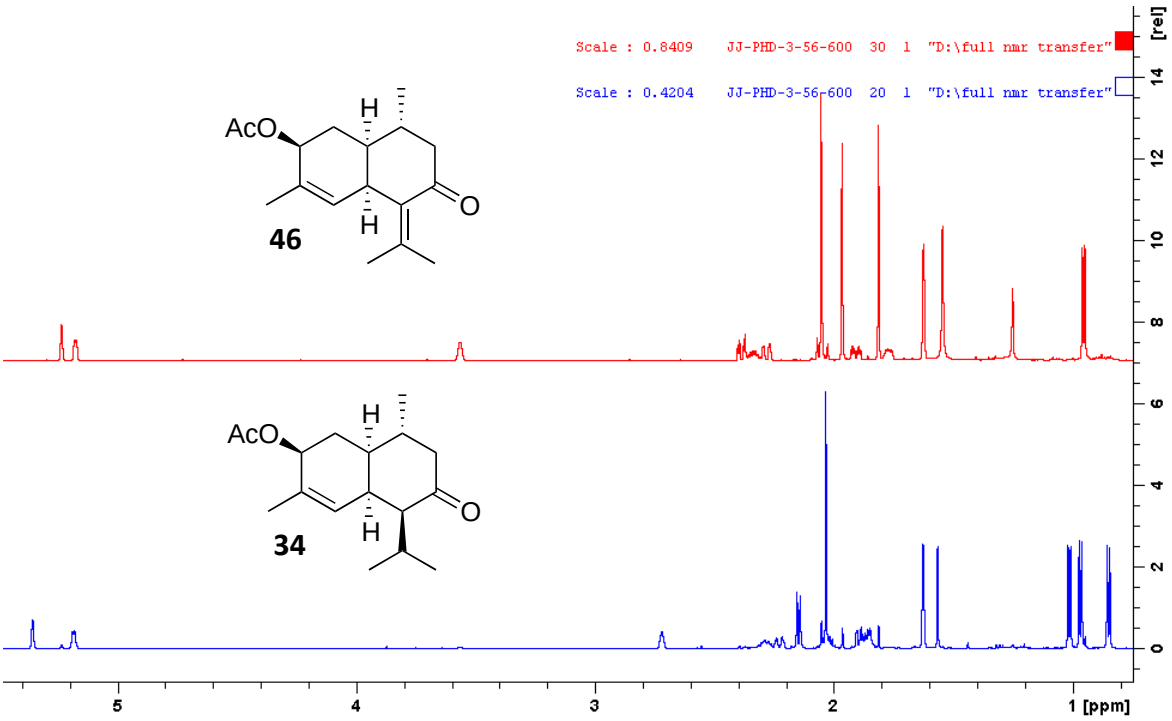


Figure 3.10 – ^{1}H spectrum of compound **34** (below) and **46** (above).

Peak 3 contained a single major component, known compound **37**,[164] which was isolated from the initial flash chromatographic run. NMR spectroscopic analysis of this compound showed a similar structure to compound **34**, with evidence that the acetoxy group was absent, replaced by a ketone. The ^{13}C NMR showed carbonyl signals at 210.8 and 198.2 ppm, consistent with the assignment of a two ketone functionalities, compared to the carbonyl signals at 214.4 and 170.9 ppm for compound **34**. The ^{1}H NMR spectrum showed three doublets at ~1 ppm consistent with the methyl groups at C-12, C-13 and C-14 all having a single adjacent proton.

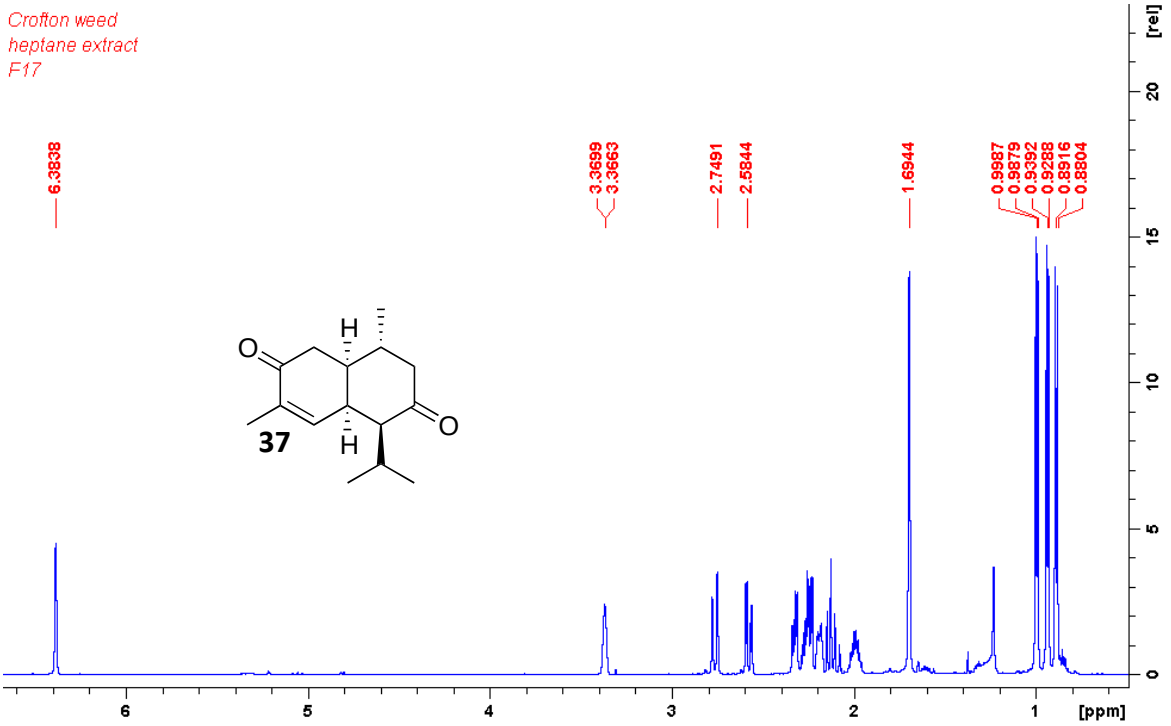


Figure 3.11 – ^{1}H NMR spectrum of compound **37**.

Peak 4, overwhelmingly the largest peak by area in the chromatogram, contained two known compounds **51**[164] and **35**,[168] which required further purification for the isolation of the components. The early eluting side of the peak contained compound **51**, which was followed by compound **35**. These compounds share the same relationships as acetoxy compounds **34** and **46**, in that they differ only by the bond order between C-6 and C-11, with compound **51** containing a single bond, and compound **35** containing a double bond in this position.

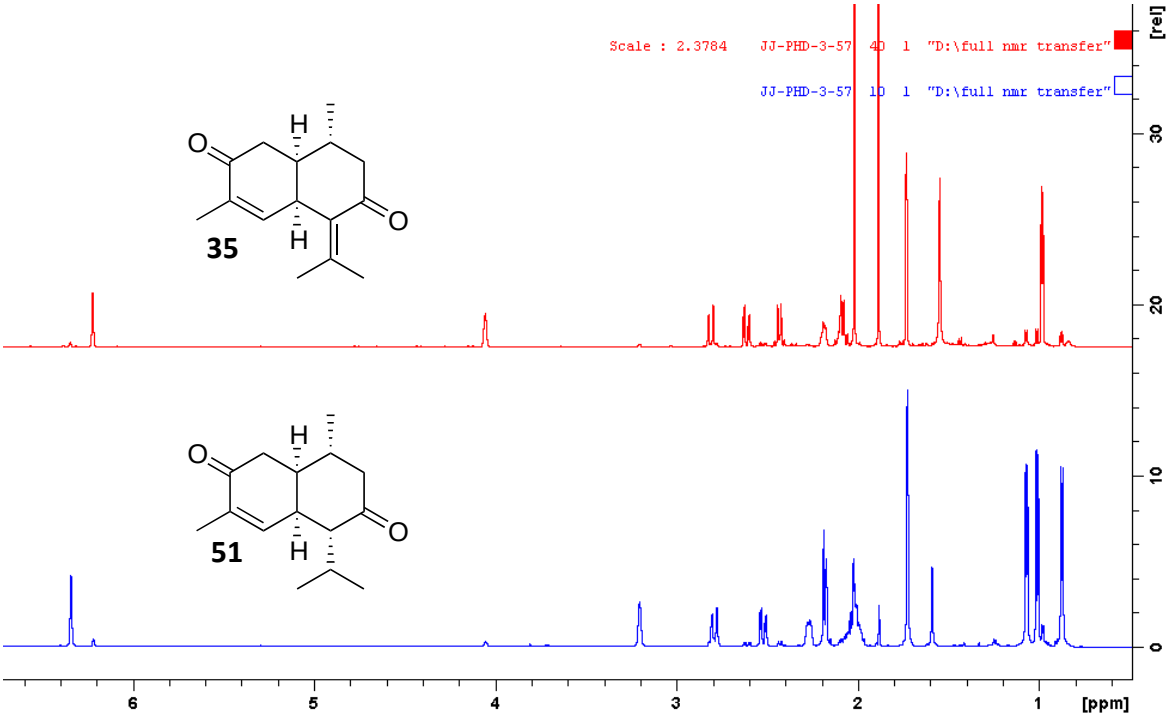


Figure 3.12 – ^{1}H spectrum of compounds **51** (below) and **35** (above).

Peak 5 contained a single major component, known compound **38**,[164] isolated in the initial flash chromatographic run. The key aspects of the NMR spectroscopic analysis for this compound were the methine signal in the ^{1}H NMR spectrum at 4.14 ppm, and the signal in the ^{13}C NMR spectrum at 67.4 ppm. These signals are consistent with the addition of a hydroxyl group at the 7-position on the ring. The single carbonyl signal at 199.6 ppm in the ^{13}C NMR spectrum is also consistent with one of the ketones being reduced to an alcohol.

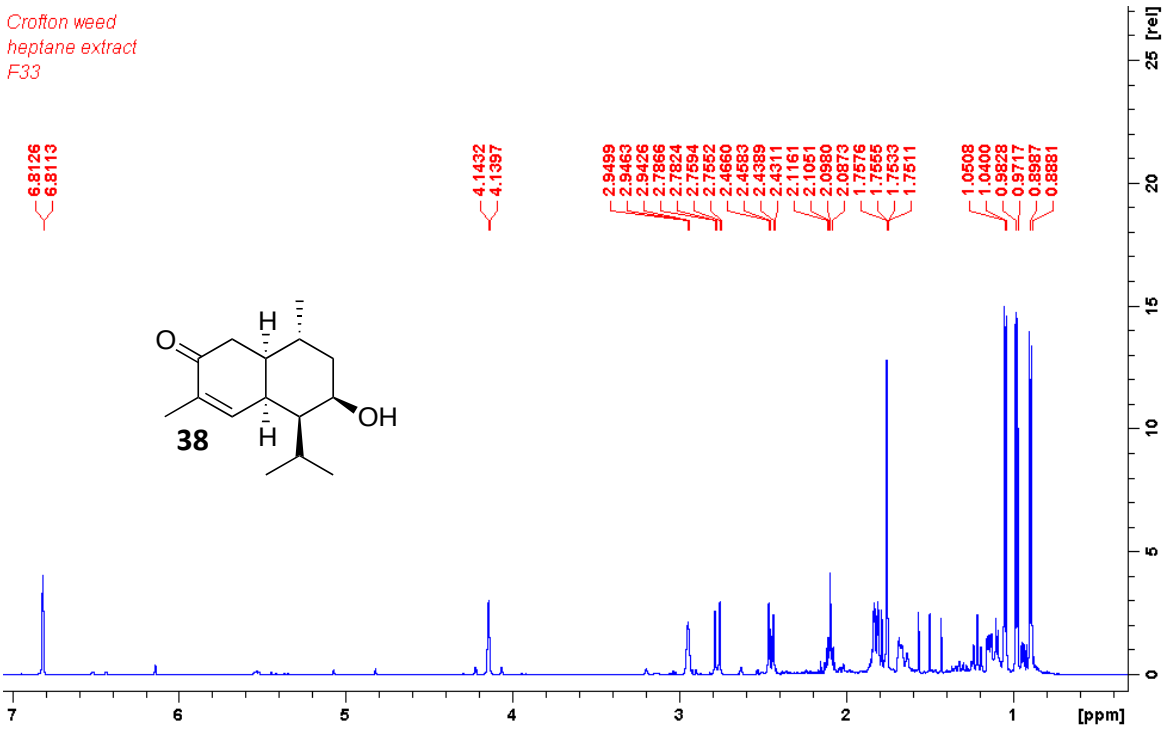


Figure 3.13 – ^{1}H spectrum of compound **38**.

Peak 6 of the separation contained a complex mixture, with a major component which required further flash chromatography for purification. The spectral data for this compound did not match with any compound identified in the literature. This compound was subsequently identified as hydroperoxide derivative **52**, primarily through reaction with PPh_3 to form known alcohol **55**, discussed below. The ^{1}H NMR spectrum showed an exchangeable singlet at 8.18 ppm, consistent with a more acidic hydroperoxide moiety being present. The terminal methylene group is supported by the signals at 5.52 and 5.61 ppm, which correlate to the same alkenyl carbon signal in the HSQCme spectrum.

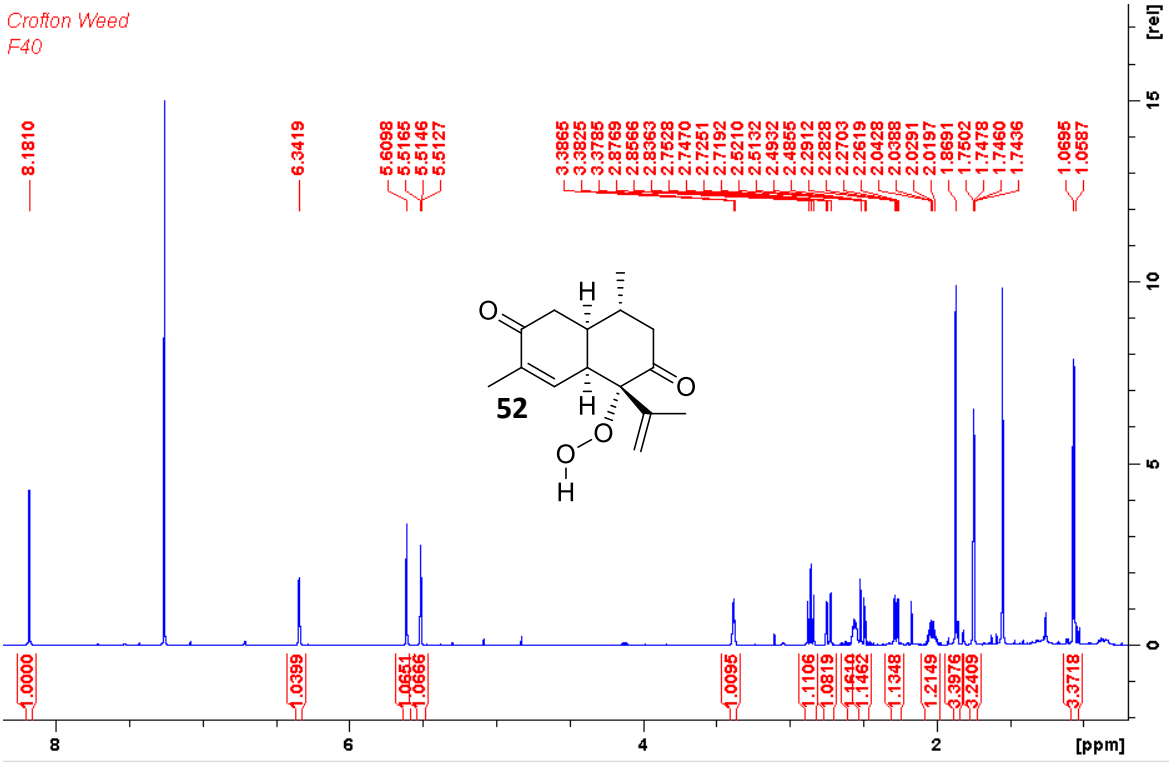
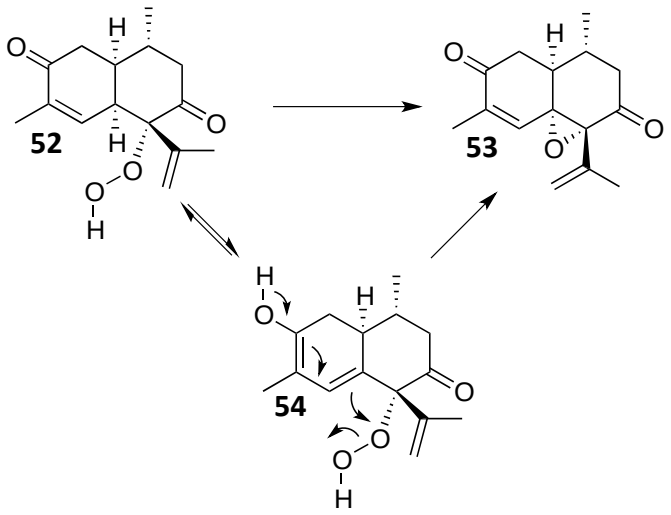


Figure 3.14 – ^{1}H NMR spectrum of compound **52**.

Another minor component was isolated during chromatographic purification of the hydroperoxide species **52**. This compound is significantly less polar than the hydroperoxide, and eluted very early in the purification. This suggests that this compound is formed as a degradation product of the hydroperoxide, rather than being part of the initial extract. The compound was identified as epoxide species **53**, which is plausibly formed from the hydroperoxide through intramolecular epoxidation of enol tautomer **54**.



Scheme 3.1 – Formation of epoxide **53** from peroxide **52**

The ^{1}H NMR spectrum for this compound shows the loss of the hydroperoxide signal at 8.18 ppm, as well as the loss of the methine signal at 3.38 ppm, which corresponded to the proton at C-5 in the hydroperoxide compound. The alkene signal at 6.01 ppm and the terminal methylene signals at 5.26 and 5.12 ppm provide evidence that these alkenes are intact in the epoxide product. The ^{13}C NMR spectrum shows signals at 70.3 and 71.8 ppm, which is consistent with the C–O bonds of the epoxide.

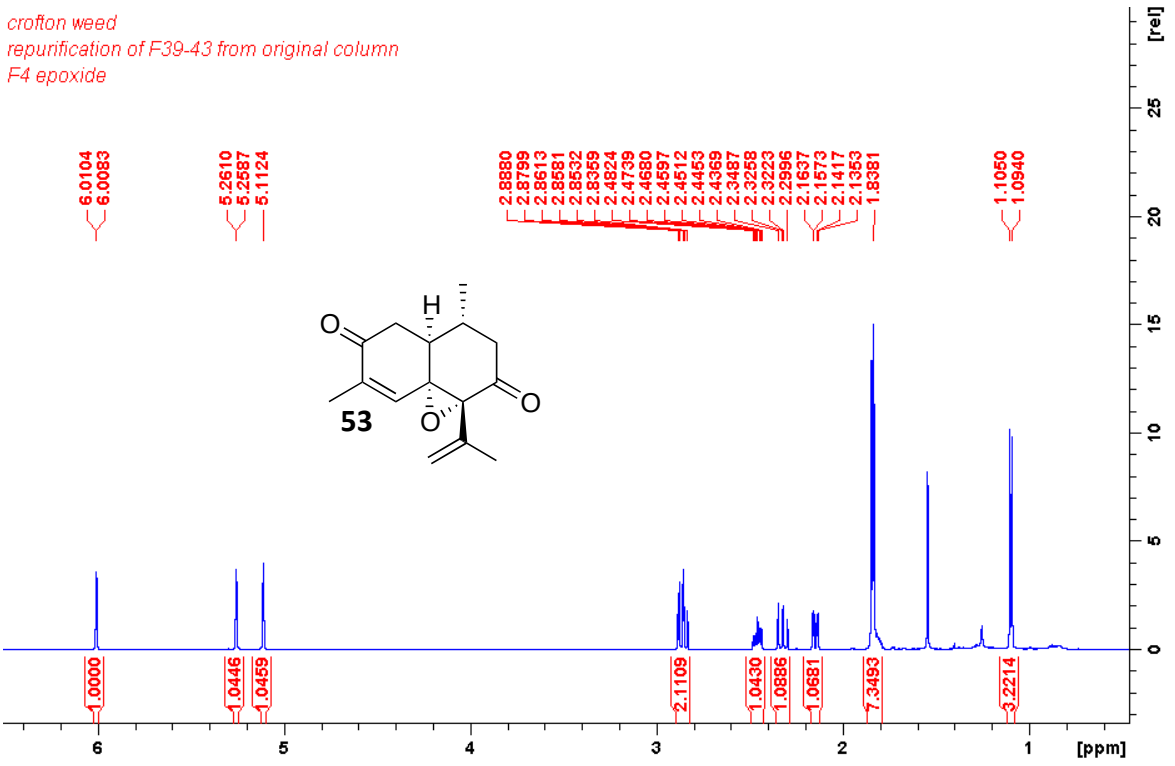


Figure 3.15 – ^{1}H NMR spectrum of compound **53**.

A chromatographic fraction from within peak 6 was observed to partially solidify. A crystal was taken and subjected to single crystal X-ray diffraction analysis and was shown to be compound **54**. This compound is particularly distinct from the major terpene derivatives, as it lacks oxygenation at the C-2 and C-7 position which is seen in all other derivatives isolated. Analysis of the ^{1}H and ^{13}C NMR spectra for the fraction that the crystal was isolated from suggest that this is only a very minor component, and was not isolated except for the single crystal.

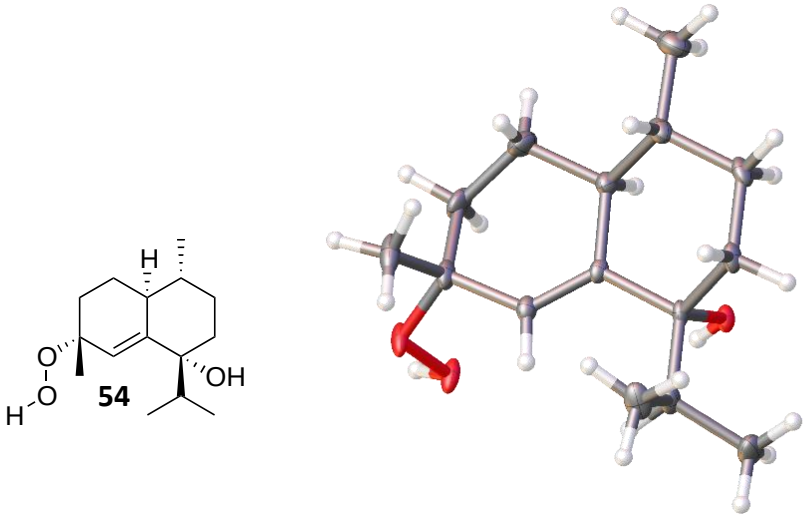


Figure 3.16 – Novel peroxide species **54** (left) and X-ray crystal structure (right)

Peak 7 appeared to contain a major component by ^{1}H NMR spectroscopic analysis, however, attempts to isolate any components by flash chromatography resulted in compound decomposition and complex mixtures of compounds obtained.

3.2.1.3 Autoxidation Studies

The isolation of hydroperoxide type compounds led to the hypothesis that the compounds isolated from *E. adenophorum* may be prone to autoxidation. To test this hypothesis, a neat sample of compound **35** was placed in a NMR sample tube in air. After 1 week, the ^{1}H NMR analysis indicated that 50% of compound **35** had been converted to hydroperoxide species **52**. This provides evidence that compound **52**, as well as other previously reported molecules may result from post-extraction autoxidation, rather than by enzymatic control of the plant. The transformation that converts compound **35** to **52** presumably occurs via an 'ene' reaction.

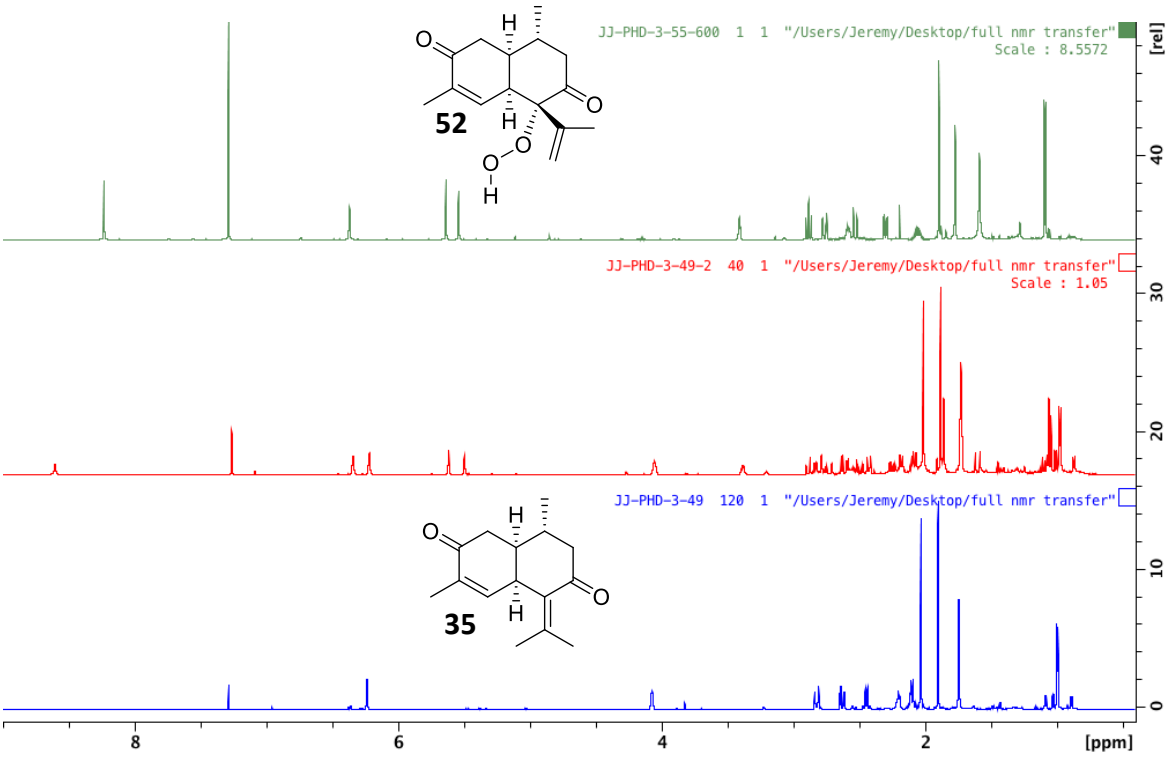
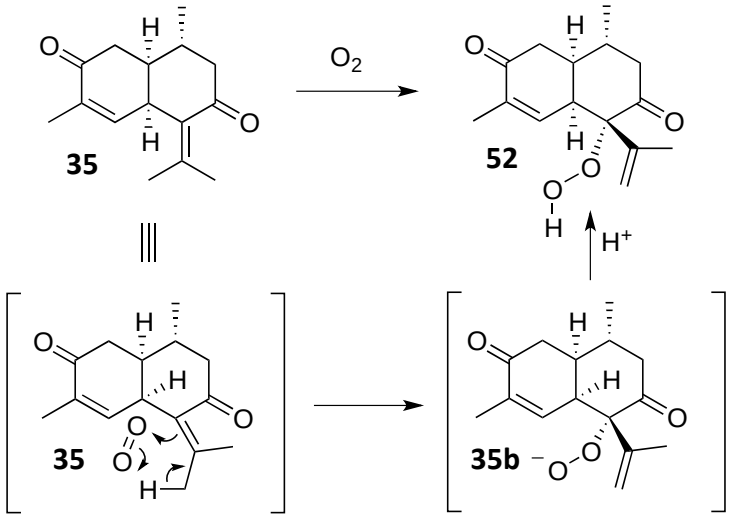


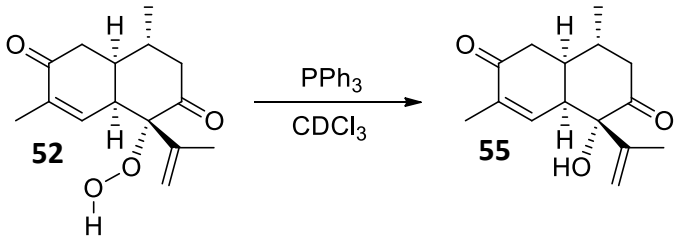
Figure 3.17 – ^{1}H NMR spectrum of compound **35** (bottom), ^{1}H NMR spectrum after 1 week in $CDCl_3$ (middle), and ^{1}H NMR spectrum of the resulting purified hydroperoxide species **52** (top).



Scheme 3.2 – Hydroperoxide **52** formation from **35** upon exposure to oxygen.

Hydroperoxide **52** has not been previously reported in the literature but corresponding alcohol **55** is known.[180] Therefore, this compound was subjected to reaction with triphenylphosphine to convert the hydroperoxide to the alcohol **55** (Scheme 3.2). The ^{1}H

and ^{13}C NMR spectroscopic data for compound **55** were consistent with those previously reported.[180]



Scheme 3.3 – Conversion of peroxide **52** to alcohol **55** through reaction with PPh_3.

This result provides compelling evidence that these cadinane compounds are prone to autoxidation, and will undergo transformations to form different components such as peroxides and epoxides. This suggests that some of the previous reports of compounds discussed in the beginning of the chapter may be describing compounds which are artefacts of isolation rather than true natural products. This is a reasonable conclusion, based on the reactivity observed directly for compound **35**, and given that many of the previously discussed isolation procedures involved laborious extraction methodologies and multiple flash chromatographic steps therefore increasing exposure to molecular oxygen.

3.2.1.4 Extraction for Quantitation and Generation of Bioactive Extracts for Testing

Based on this observed reactivity, a subsequent extraction and isolation where the extract was treated more carefully was undertaken. The sample was chilled immediately after the extraction and was stored in the fridge, away from light, and under a nitrogen atmosphere where possible. The chromatographic parameters were kept constant between the two runs, and this can be seen in the similarities between the two extraction profiles. This second chromatogram shows much smaller peaks after peak 5. Specifically, compounds **52**–**54** from peak 6, and peak 7 from the first extraction were not present. This result provides further evidence that this PHWE method provides a means of efficiently extracting sensitive compounds due to the short extraction times, and in this instance, limiting exposure to molecular oxygen.

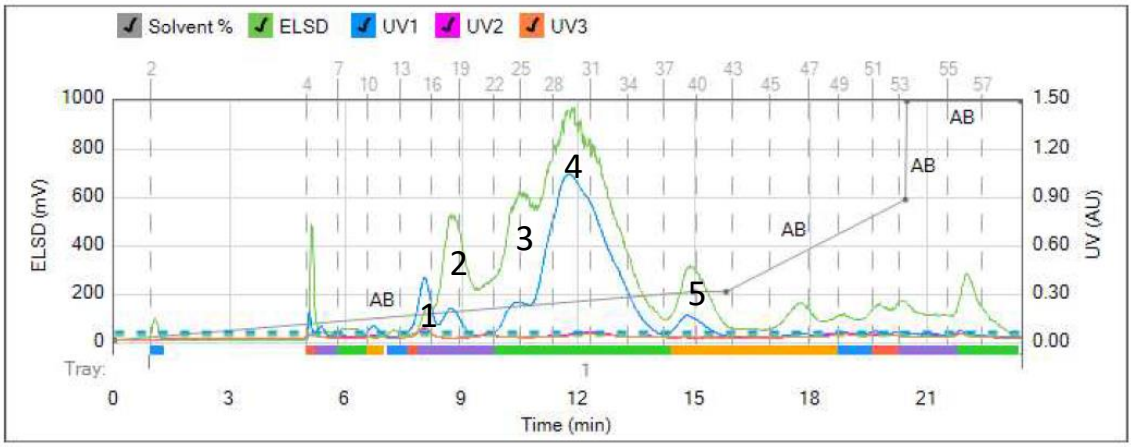


Figure 3.18 – Separation profile of fresh *E. adenophorum* heptane extract, major peaks are numbered 1 through 5.

The following chromatogram, zoomed in from Figure 3.18 above, shows which compounds were isolated from which sections of the separation profile. These samples were quantified as individual compounds or mixtures as appropriate to get a more accurate picture of the yield of each compound from the plant.

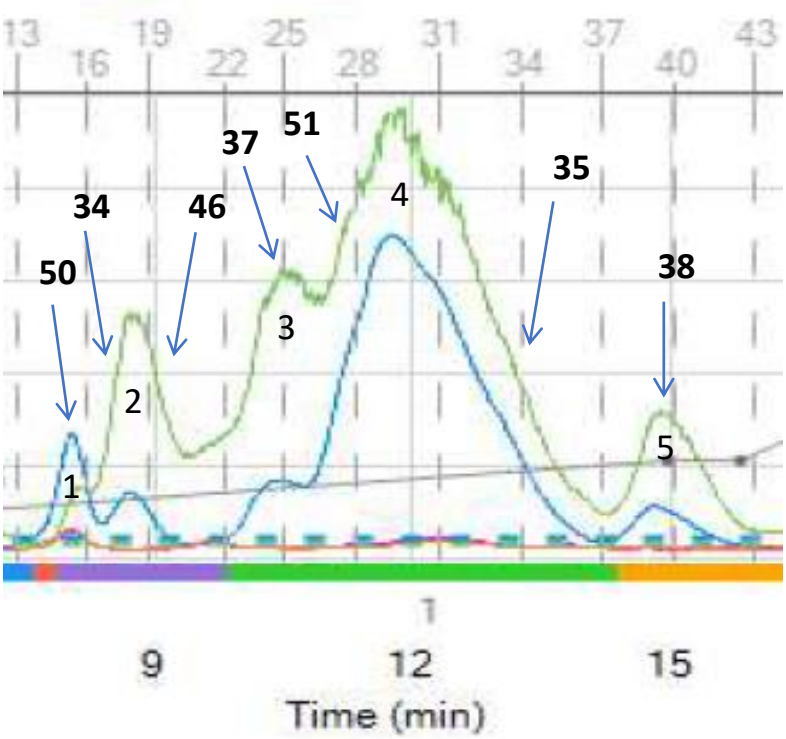


Figure 3.19 – Expansion of chromatogram (Figure 3.18) of *E. adenophorum* heptane extract without oxidation products present.

Compound	Yield (mg)	Yield (% w/w)
50	4	0.01
34, **46**	77	0.21
37, **51**, **35**	428	1.14
38	43	0.11
Total (excl **50**)	548	1.46

Table 3.2 – Compounds and mixtures isolated from *E. adenophorum.* Compound **50** was excluded from the total yield, as the cadinane terpenes were the compounds of interest.

The results obtained for the extraction of this plant suggest that the extraction method, and subsequent handling and purification of the extract is of critical importance. The combined yield of compounds **37**, **51** and **35** was nearly double (1.14 % vs 0.65 % w/w) in the second extraction, due to these factors. The demonstration that compound **35** can be converted to a hydroperoxide, and subsequently into an epoxide species through reaction with molecular oxygen suggests these compounds must be stored carefully. This result also provides further evidence that many of the minor compounds previously reported may be artefacts of the extraction and isolation of the plant, and not true natural products. Of course, these oxidation products may be the source of the allelopathic effects shown by *E. adenophorum*, but further study is required to determine this as previous studies of this activity have primarily focussed on the more abundant metabolites such as **37**, **35** and **51**.

3.2.1.5 Extraction and Isolation of Ethyl Acetate Soluble Components

The residual aqueous extract was further extracted with EtOAc, which showed a distinctly different ^{1}H NMR spectral profile compared with the heptane extract, which suggested a different class of compounds was being extracted with the EtOAc, with no cadinane terpenes observed. Of note was the appearance of a number of signals above 6.5 ppm, which were absent in the heptane extract.

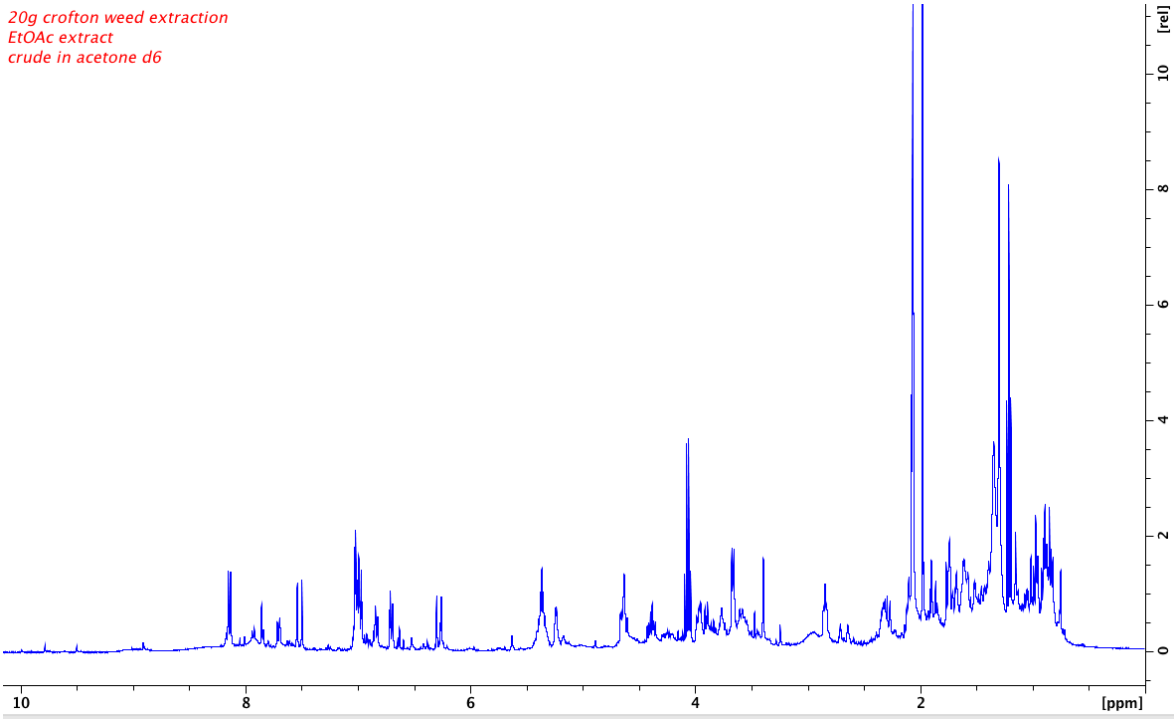


Figure 3.20 – ^{1}H NMR spectrum of the EtOAc extract of *E. adenophorum*

The ^{1}H NMR spectrum and TLC analysis of this extract provided evidence of two major components. Normal phase silica gel column chromatography yielded co-elution of the two compounds, however, they were able to be separated successfully using reversed-phase flash column chromatography to provide compounds **56** and **57** in 0.18 % and 0.22 % yield w/w respectively. The ^{1}H and ^{13}C NMR spectroscopic data for these compounds was consistent with a report by He and co-workers in 2016.[185]

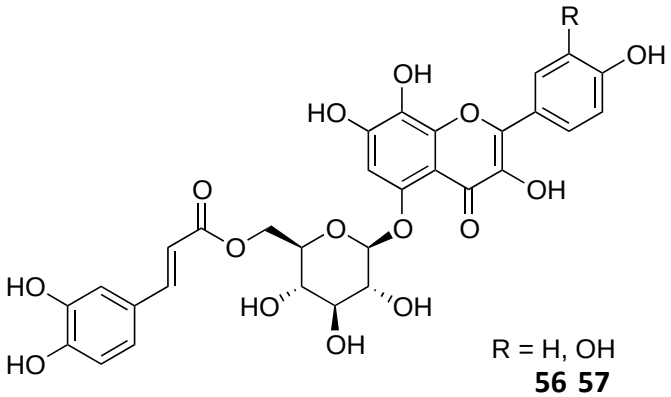


Figure 3.21 – Glycosides **56** and **57** isolated from *E. adenophorum* EtOAc extract.

3.2.2 Pyrethrins from Dalmatian Chrysanthemum (*Chrysanthemum cinerariaefolium*)

3.2.2.1 Background

Chrysanthemum cinerariaefolium (L.) (Dalmatian chrysanthemum, Pyrethrum) is a member of the daisy (Asteraceae) family and contains a mixture of six insecticidal compounds, collectively known as the pyrethrins. These are separated into classes I and II as shown in Figure 3.22. The focus of the extraction of the pyrethrins was to test PHWE for extraction of thermally sensitive molecules, as pyrethrins are known to be thermally labile. If successful, the subsequent goal was to develop a method for the selective hydrogenation of pyrethrin I and pyrethrin II to convert them selectively into lesser abundant jasmolin I and jasmolin II.

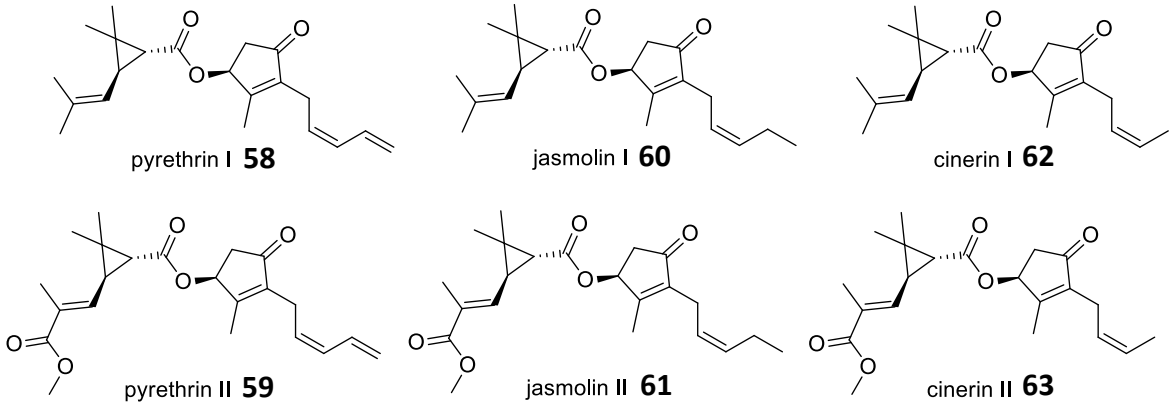
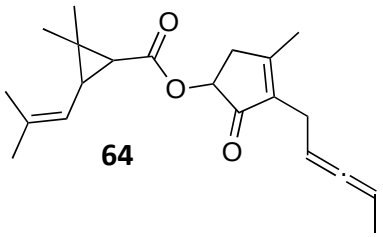


Figure 3.22 – The major components of pyrethrin extract

The structure of pyrethrin I was initially reported by Staudinger in 1924[186] as compound **64**, and methods for the quantitative isolation of the primary active components were published in the late 1920's.[187] In Staudinger's initial report, the terminal double bond of the side chain was incorrectly placed in the adjacent position, forming an allene, and lacked a double bond in the cyclopentenone ring. Further, the minor components, cinerin I and II and jasmolin I and II were not isolated and unambiguously identified until 1944/45[188,189] and 1966[190] respectively.

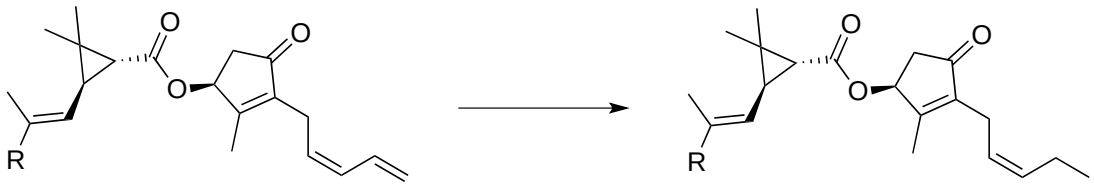


64

Figure 3.23 – 1924 proposed structure of pyrethrin I

The quantity of the pyrethrins within the plants is highest in the flowers, and continues to increase in concentration as the flowers mature. Specifically, the achene (small fruit containing the seed) of the flower contains ~90% of the pyrethrins, but only accounts for ~30% of the weight of the flower. Subsequently, it was found that the pyrethrins are found in oily deposits on the surface of the achenes. For practical reasons it is most convenient to take the whole flowers for isolation of these compounds.[191]

Pyrethrin I and II are the most abundant and most active components of this mixture in the toxicity towards insects.[192,193] They are, however, also the least stable and will rapidly undergo degradation due to oxygen and light when used as an insecticide.[194] This is a desirable property for many applications because it means that they do not linger in the environment and contaminate food crops. However, due to this instability, it may be beneficial to take crude pyrethrum extract and convert the pyrethrin I and II selectively into jasmolin I and II to create an insecticidally active but more stable extract.



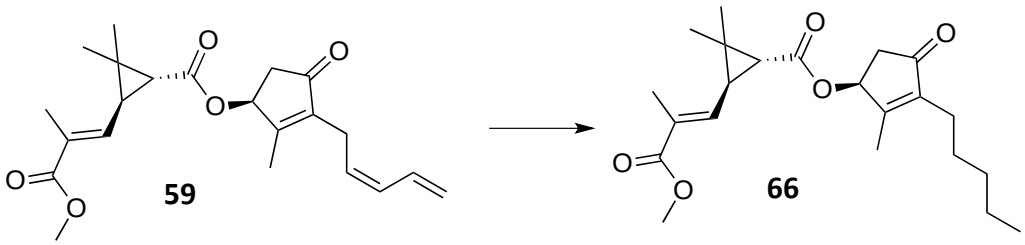
58 pyrethrin I R = CH_3
59 pyrethrin II R = CO_2CH_3

60 jasmolin I R = CH_3
61 jasmolin II R = CO_2CH_3

Scheme 3.4 – Formation of jasmolin I (**60**) and II (**61**) from pyrethrin I (**58**) and II (**59**).

There are currently no reports of this transformation, as previous attempts proved unsuccessful. Traditional metal catalyst based hydrogenation were inappropriate due to the lack of selectivity in hydrogenation of the molecule, and indeed cleavage of ester linkage, presumably by formation of a π-allyl cation.[186,195] A report of the hydrogenation using PtO_2 as the metal catalyst on pyrethrin II with a short reaction time was found to

yield dihydrojasmolin II (**66**), where the side chain was fully saturated.[196] A longer reaction time resulted in cleavage of the ester portion. Formation of the dihydro derivatives is not desirable for insecticidal applications as they are not insecticidally active.[197]



Scheme 3.5 – Hydrogenation of pyrethrin II with PtO_2

For the development of a method for the selective hydrogenation of pyrethrin I and II to form jasmolin I and II, it was chosen to investigate using diimide as the reducing agent. Diimide (H_2N_2) is a mild hydrogenation reagent,[198] which is ideal for the selective hydrogenation. Diimide reductions were observed as early as 1905 when glyceryl oleate was converted to stearic hydrazide[199] which was published alongside the conversion of oleic acid to stearic acid in the presence of hydrazine in 1929.[200] Diimide was not implicated in the process until the 1960's.[199] Previous reports have shown that diimide reductions proceed most efficiently for lesser substituted alkenes, and do not proceed for tri or tetra-substituted alkenes.[199] These reductions are also known to proceed faster for symmetrical multiple bonds (C=C, N=N) than for polarised multiple bonds such as C=O or C=N.[198]

Pyrethrin I and II are a great model for testing development of a selective hydrogenation method as they contain mono, di, tri, and tetrasubstituted C=C double bonds. Given the previously reported lack of reactivity towards highly substituted alkenes, the proposed major products of the reaction of pyrethrin I and II with diimide would be jasmolin I and II following hydrogenation of the terminal alkene. Pyrethrin I and II are the only components of the mixture with a mono-substituted alkene, so the reaction should proceed most rapidly for these components. The competing reaction, where hydrogenation occurs on the disubstituted alkene present in the jasmolins and cinerins would form dihydrojasmolin I and II, and dihydrocinerin I and II (**65**–**68**).

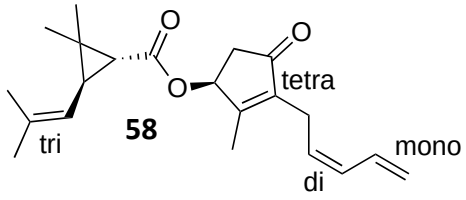
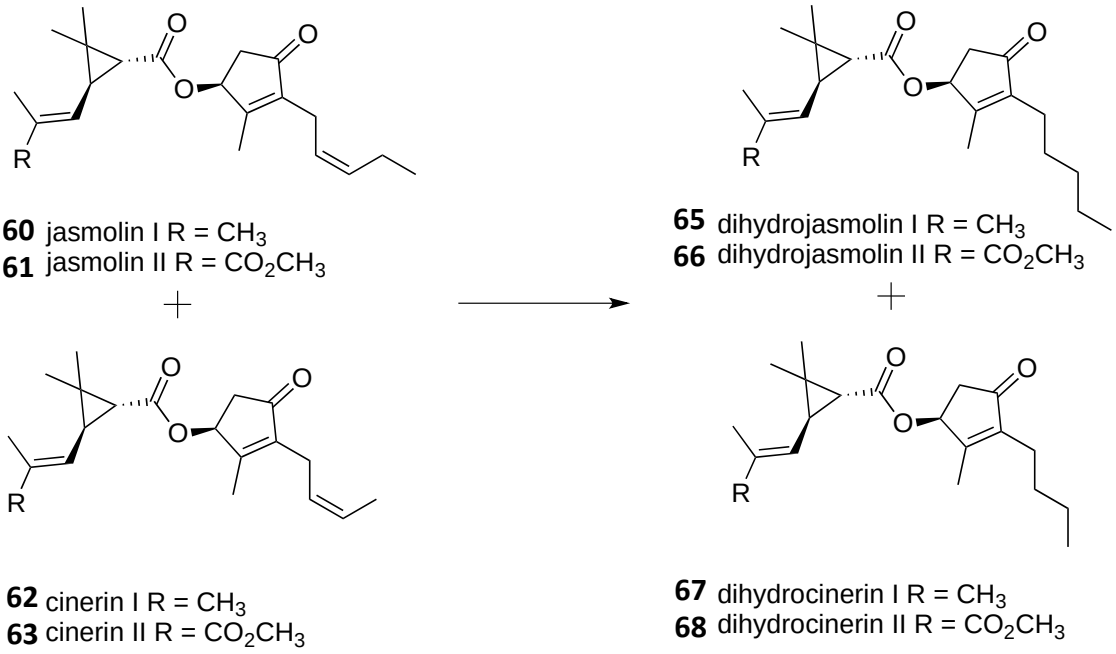


Figure 3.24 – Substitution pattern of the alkenes in pyrethrin I (**58**)

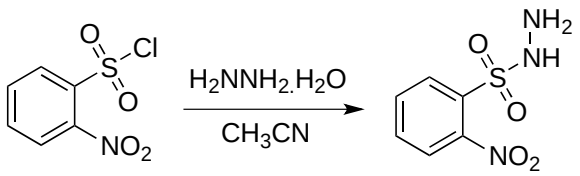


Scheme 3.6 – Competing reaction to form dihydro derivatives **65**–**68**.

Though the approximate amount of pyrethrins in the crude extracts can be determined, there is an added complication of using diimide. Diimide must be generated *in situ* as it is prone to disproportionation to form nitrogen gas and hydrazine, as well as decomposition to nitrogen and hydrogen gas during the reaction. This makes the addition of a stoichiometric amount of reagent difficult to achieve full conversion.[199] There are numerous reported methods for the *in situ* generation of diimide, however, many of these involve the use of molecular oxygen as a reagent.[199] A method based on ease of operation as well as a method that did not rely on molecular oxygen was chosen, as pyrethrin degrades in air.[194]

A report from 2002 details the first use of NBSH (2-nitrobenzenesulfonyl hydrazide, **70**) to generate diimide for the reduction of alkenes[201], which employs a modification of a preparation of the compound reported by Myers and co-workers in 1997.[202] A more recent report by Marsh and co-workers again demonstrates the use of NBSH as a reagent

for the efficient generation of diimide under mild conditions with the reduction of a number of alkenyl substrates.[203]

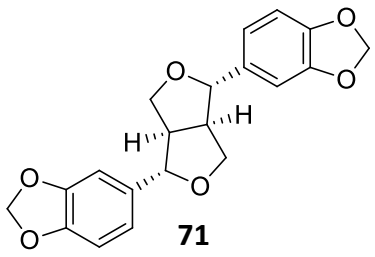


69 **70**

Scheme 3.7 – Synthesis of *o*-nitrobenzenesulfonylhydrazide (NBSH)

3.2.2.2 Extraction and Analysis of the Crude Extract

Pyrethrum flower obtained from Botanical Resources Australia contained BHT to supress oxidative decomposition of pyrethrins **58** and **59**. For reaction experiments, plant material was extracted in 15 g batches using 200 mL 35% v/v EtOH:H_2O. Subsequent extraction with heptane yielded the crude pyrethrin extract. The extraction was found to be highly reproducible, with the process repeated multiple times with very similar results for the mass of the crude extract (~1.6% w/w) and components observed by ^{1}H NMR spectroscopic analysis. NMR spectra for these extracts showed the primary identifiable components as the pyrethrins **58**–**63**, as well as the compound sesamin (**71**). The key signals in the ^{1}H NMR spectrum of the crude extract are 3 of the alkene signals for pyrethrin I and II, shown in Figure 3.26. Enrichment in pyrethrin I and II supports the mild nature of the method, and demonstrates again the viability of the method for the extraction of sensitive substrates.



71

Figure 3.25 – Sesamin

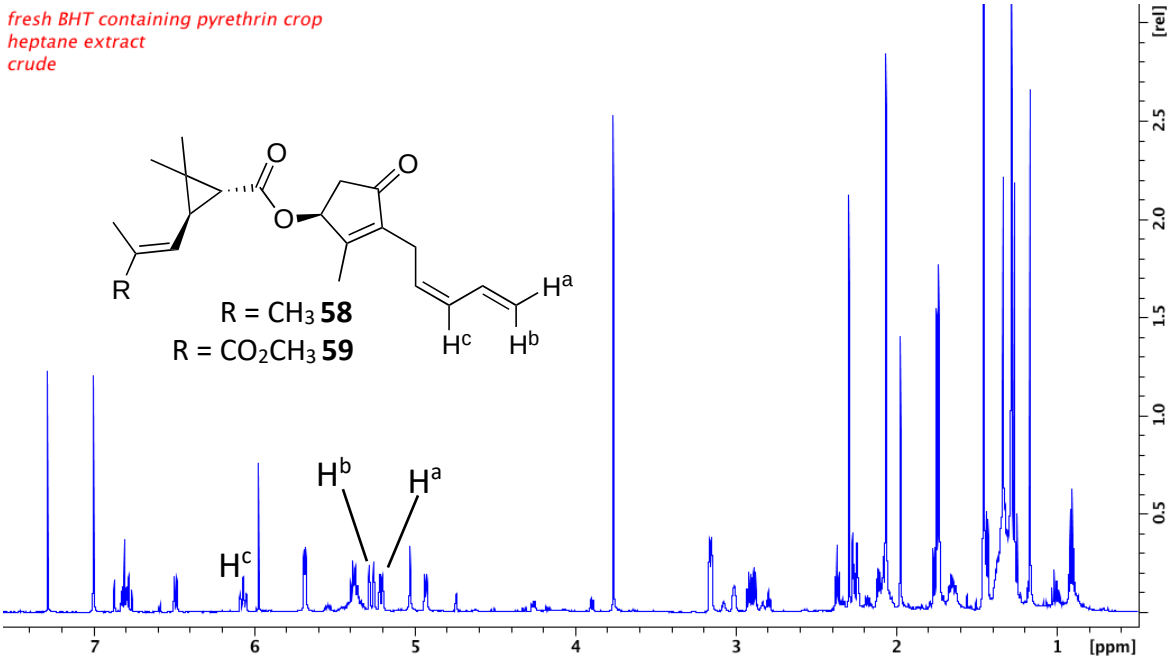
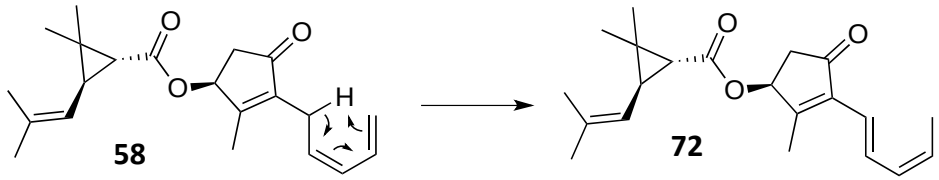


Figure 3.26 – ^{1}H NMR spectrum of the heptane extract of *C. cinerariaefolium.*

NMR spectroscopic analysis alone is insufficient for determination of the relative proportions of the compounds in the extract due to their structural similarities and therefore overlapping signals in the analysis. For this reason, NMR spectroscopic analysis was used in combination with GC-MS for the analysis of extracts and reaction mixtures. LC-MS would be preferable, however an appropriate instrument was unavailable at the time. Pyrethrin I and II are not stable to GC-MS injection conditions, as they undergo thermal isomerisation to form isopyrethrin I and II.[204,205]



Scheme 3.8 – Thermal isomerisation of pyrethrin I (**58**) to give isopyrethrin I (**72**)

In the GC-MS trace of the crude extract, there were two peaks with mass of pyrethrin I, which have mass spectra consistent with pyrethrin I (**58**) and isopyrethrin I (**72**). The jasmolins **60** and **61** and cinerins **62** and **63** are not susceptible to this degradation and are unchanged in the GC-MS analysis. The ratio of jasmolins to cinerins is ~0.6:1, showing that the jasmolins are the least abundant component of the crude extract. The combination of pyrethrin I and II can be measured in the ^{1}H NMR spectrum due to the

unique alkene signals shown in Figure 3.26. Therefore, given that the primary goal was to react the majority of the pyrethrin I and II to form jasmolin I and II, it is valid to first take an NMR spectrum of the sample to determine residual pyrethrin I and II levels, and subsequently subject the sample to GC-MS analysis to determine the ratios of the jasmolins and cinerins. Hence, GC is not the most appropriate method to quantify pyrethrin I and II. However, as the interest was in relative ratios, and conversion to jasmolin I and II, it was the most convenient method.

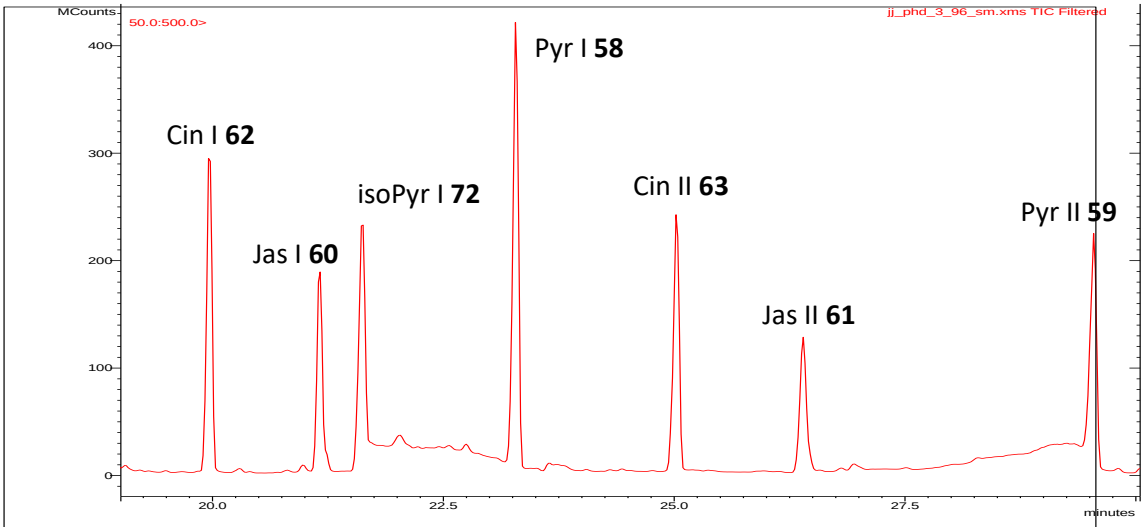
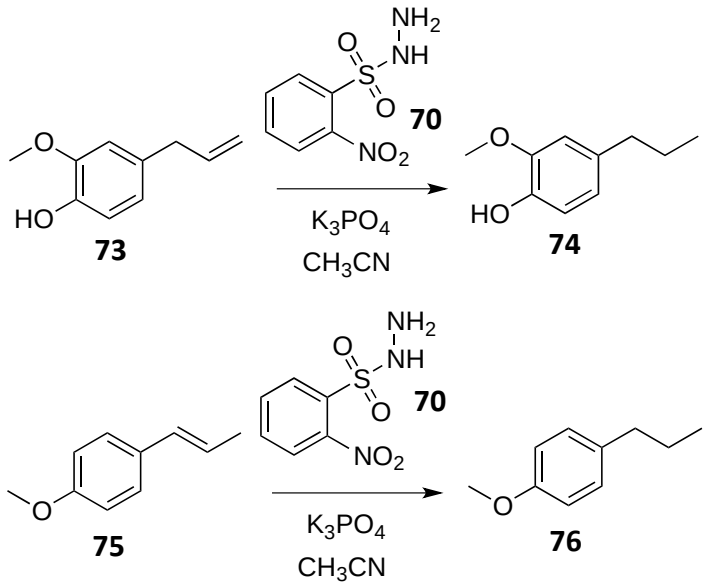


Figure 3.27 – Partial GC chromatogram of crude pyrethrin heptane extract.

3.2.2.3 Synthesis and Testing of NBSH

For reaction with pyrethin extract, NBSH (**70**) was synthesised through further modification of the reported method. Following reaction by the conditions of Marsh and co-workers, the compound was precipitated through addition of water, and collected by filtration, which resulted in the rapid generation of gram-scale quantities of this reagent. Following the synthesis of this reagent, 2 concurrent test reactions were performed to investigate the differences in reactivity of 2 simple alkenes. Conditions were based on the method of Marsh.[203] Eugenol isolated from cloves (*S. aromaticum*), and anethole isolated from Chinese star anise (*I. verum*) were used as test substrates for confirming the reactivity of the reagent. These were chosen because they are both phenylpropenes which differ in the position and substitution of the alkene.



Scheme 3.9 – Reaction of eugenol (above) and anethole (below) with NBSH

Eugenol and anethole were separately reacted with 1 equivalent of NBSH in acetonitrile. An aliquot of each reaction was taken at 1 h and the ratio of starting material to product determined by integration ratio of the appropriate signals in the ^{1}H NMR spectra. The eugenol reaction had proceeded to ~20 % completion, whereas the anethole reaction, which was expected to be much slower as it contains di-substituted alkene had only proceeded ~5%. This provided evidence that the reagent can reduce C=C bonds under the given conditions, and that the reaction with a mono-substituted alkene proceeds more rapidly. The reactions were worked up after a further 19 h of stirring. At this point, the progress of each reaction had increased to ~60 % for the eugenol reaction and ~51 % for the anethole reaction. This result showed that the reagent behaves as expected and the generated diimide undergoes other reactions such as disproportionation, meaning the reaction does not proceed to completion with 1 equivalent of NBSH, and provided information for how much reagent may be required for a selective alkene reduction. The ^{1}H NMR spectroscopic data obtained for this reaction is shown in the ^{1}H NMR spectrum below of the 1 h aliquot taken of the reaction with eugenol (**73**). The appearance of the signals for the saturated side chain is clear as shown on the spectrum.

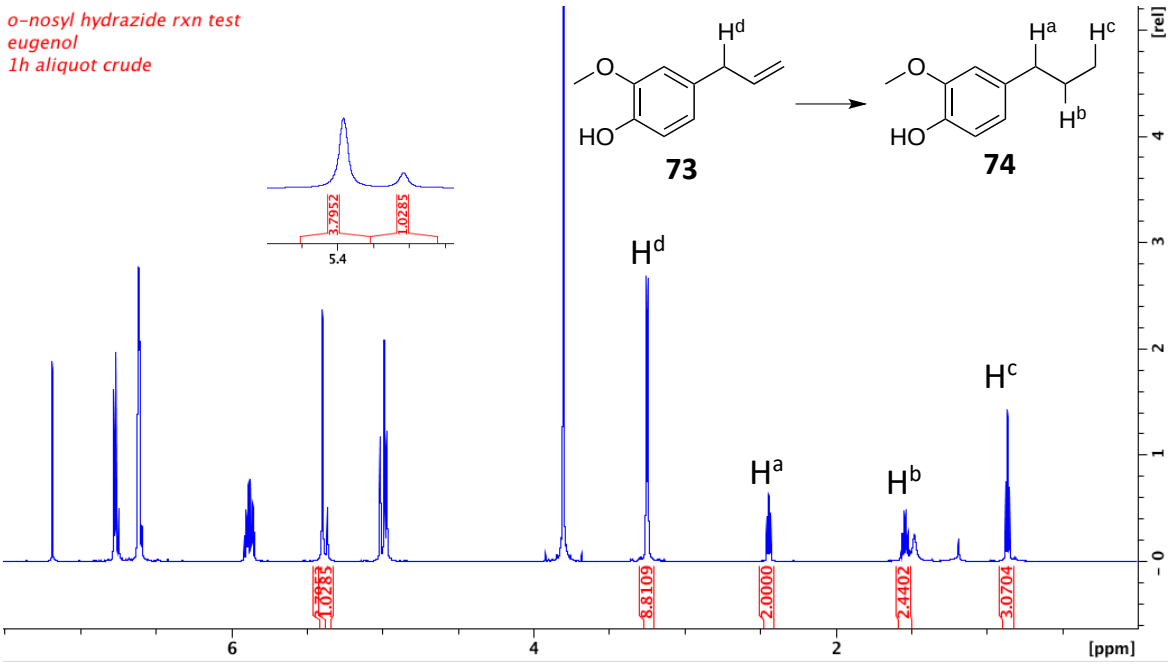


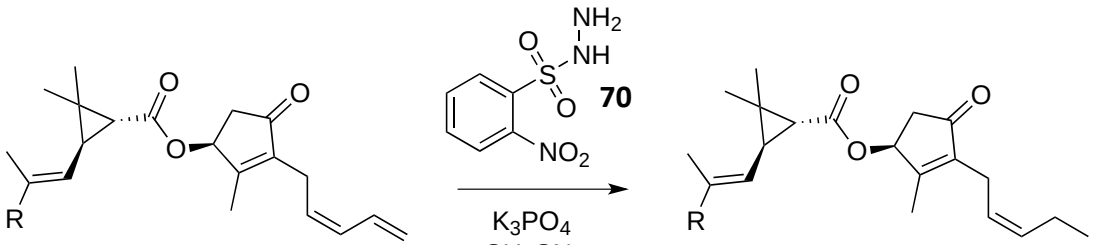
Figure 3.28 – ^{1}H NMR spectrum of the reaction of eugenol with NBSH after 1 h.

3.2.2.4 Hydrogenation of Pyrethrin Extract with NBSH

The primary goal of the diimide reduction reactions was to form an extract that was enriched with jasmolin I and II, where there was negligible pyrethrin I and II and minimal formation of dihydrojasmolin I and II. A separate experiment was also conducted, specifically targeting the dihydro derivatives. The degree of enrichment was determined by the ratio of jasmolins to cinerins compared with the crude extract. An optimisation study was carried out due to the previously discussed difficulties in performing this reaction stoichiometrically. The results from the optimisation of conditions for diimide reduction of the pyrethrins are summarised in Table 3.3.

Entry	Crude pyrethrins (mg) from 45 g plant	NBSH (mg)	Time (h)	Ratio Jasmolins to Cinerins	Ratio natural to dihydro derivatives	Crude yield (mg)	Isolated Yield (mg, %w/w)
Crude extract	-	-	-	0.6:1	-	-	-
1	590	659	21	4:1	1:1	500	297 (**60**–**63**) 0.66%
2[a]	722	400[a]	18 76	1.6:1 5.2:1	- 2.3:1	- 546	-[b] -
3	721	250	42	4.0:1	10.4:1	564	309 (**60**–**63**) 0.69%
4	700	800	72	-	-	429	170 (**65**–**68**) 0.38%

Table 3.3 - Results from diimide reduction tests. [a] - 250 mg of NBSH was added at t = 0, followed by an extra 150 mg after 18 h. [b] - As the reaction had proceeded further than desired, the mixture of products (**60**–**63**, **65**–**68**) were not chromatographed in this case.



58 pyrethrin I R = CH_3
59 pyrethrin II R = CO_2CH_3

60 jasmolin I R = CH_3
61 jasmolin II R = CO_2CH_3

Scheme 3.10 – Diimide mediated selective hydrogenation of pyrethrins to jasmolins

The initial attempt at the selective hydrogenation (Table 3.3, Entry 1) indicated by GC-MS and ^{1}H NMR spectroscopic analysis that all the pyrethrins had been either reacted or degraded, and that there was a significant enrichment of the jasmolins in the sample. The ^{1}H NMR spectrum clearly shows the loss of the alkene signals which are distinct for pyrethrin I and II (see Figure 3.26).

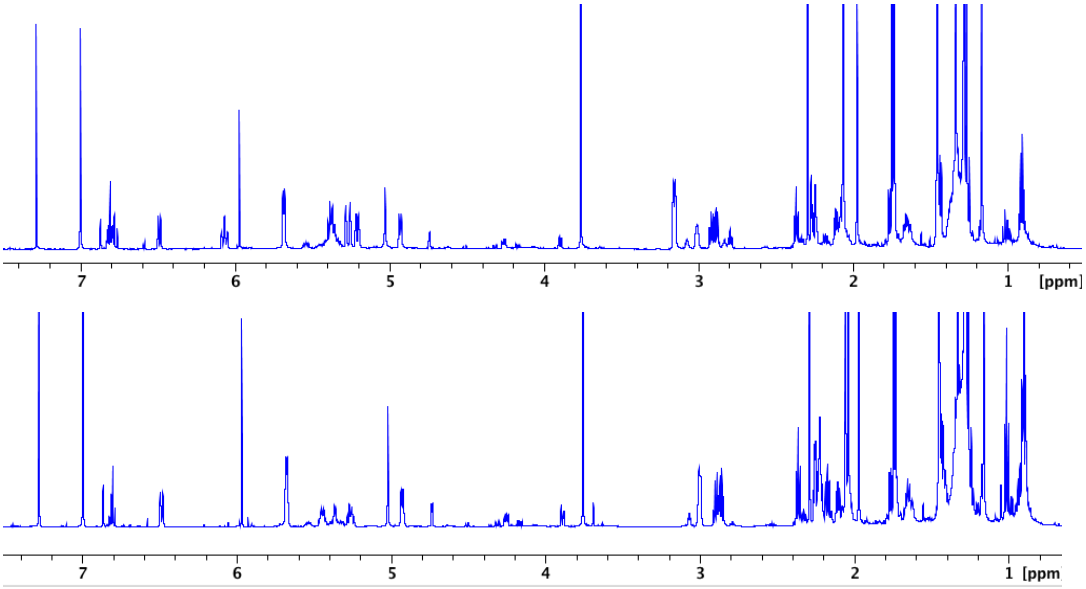


Figure 3.29 – ^{1}H NMR spectrum of initial diimide reduction of pyrethrin extract (below) vs. the crude pyrethrin extract (above).

The reaction was subsequently fractionated by automated flash column chromatography into 2 separate fractions, one containing the I series and the second containing the II series. GC-MS analysis of these fractions showed each contained 4 major components. The ratio of total jasmolins to total cinerins was ~4:1 and the ratio of the desired product to the dihydro products was ~1:1. This shows significant enrichment of jasmolin content which provides evidence that the hydrogenation protocol was proceeding as intended. The yields of this reaction were 114 mg I series and 183mg II series from 45g of pyrethrum crop representing a yield of 0.66% w/w pyrethrins.

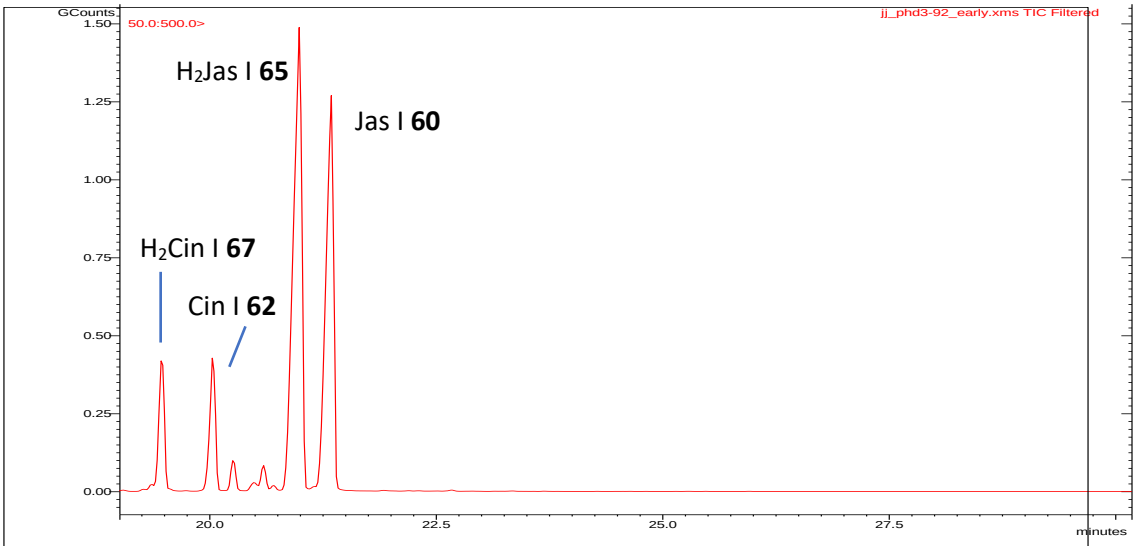


Figure 3.30 – GC chromatogram of diimide reduction Table 3.3, entry 1, I series

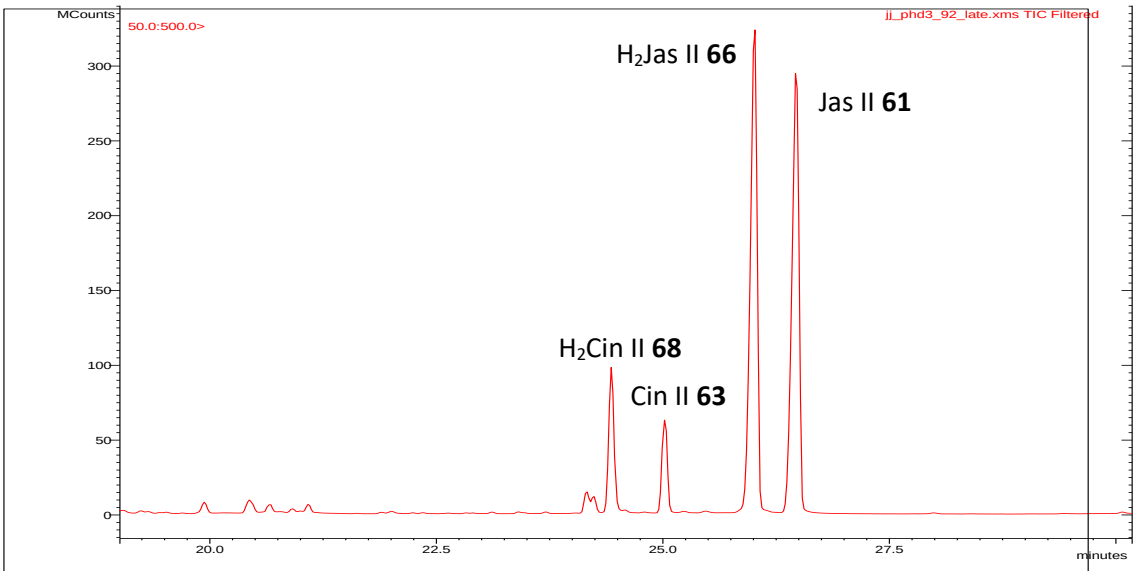


Figure 3.31 – GC chromatogram of diimide reduction Table 3.3, entry 1, II series

Entry 2 shows a reduction protocol which initially showed enrichment of jasmolins **60** and **61** with significant quantities of the pyrethrins **58** and **59** still present, which upon further reaction showed complete reaction of pyrethrins, but with significant levels of the undesired dihydro products **65**–**69**. Entry 3 shows formation of an extract enriched in the jasmolins and with minimal amounts of the over hydrogenated jasmolins and cinerins present, which fulfils the goal of achieving an extract which is enriched in the jasmolin components, with minimal formation of the dihydro derivatives.

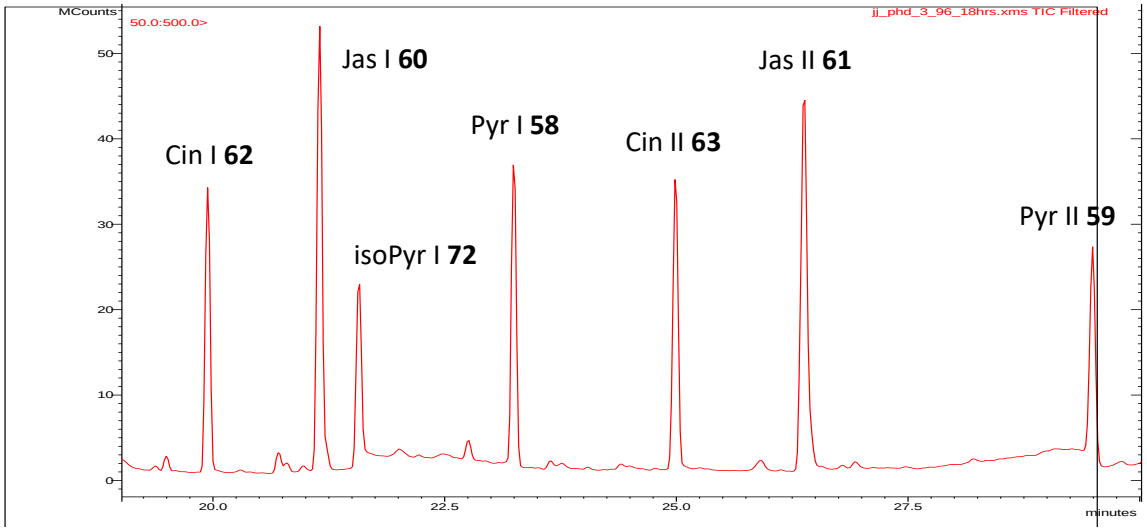


Figure 3.32 – GC chromatogram of diimide reaction Table 3.3, Entry 2, t = 18 h.

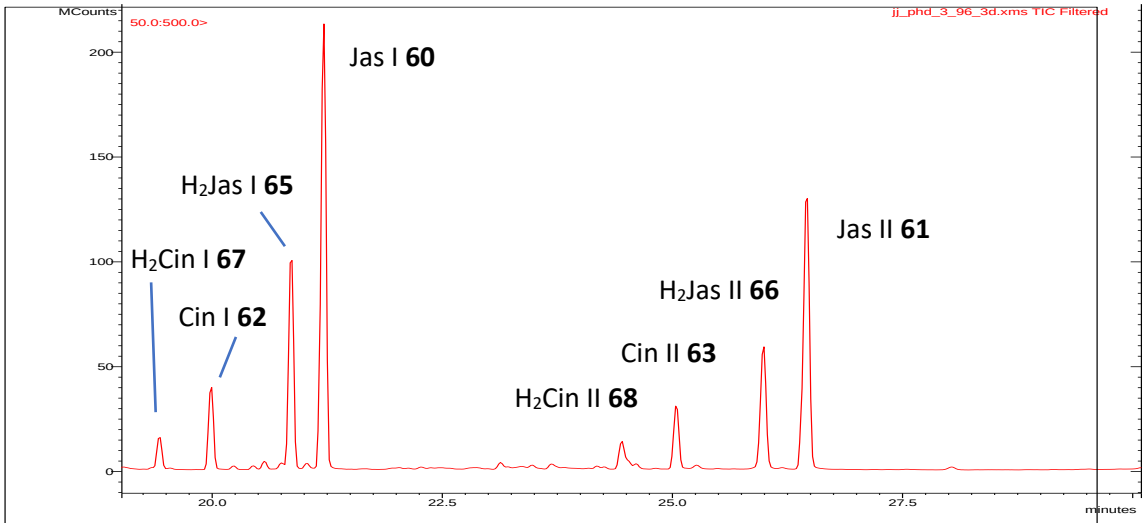


Figure 3.33 – GC chromatogram of diimide reaction Table 3.3, Entry 2, t = 76 h.

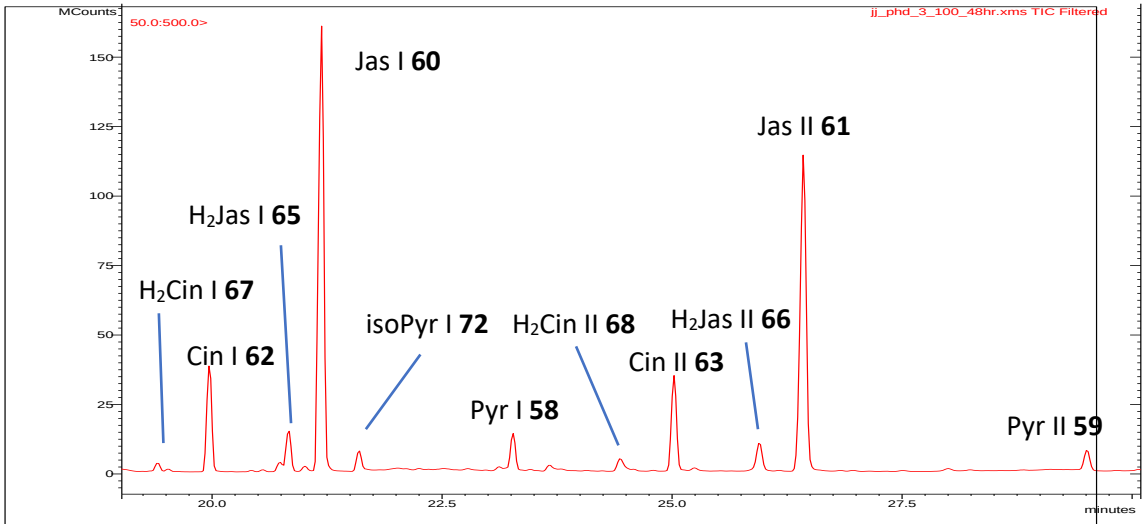
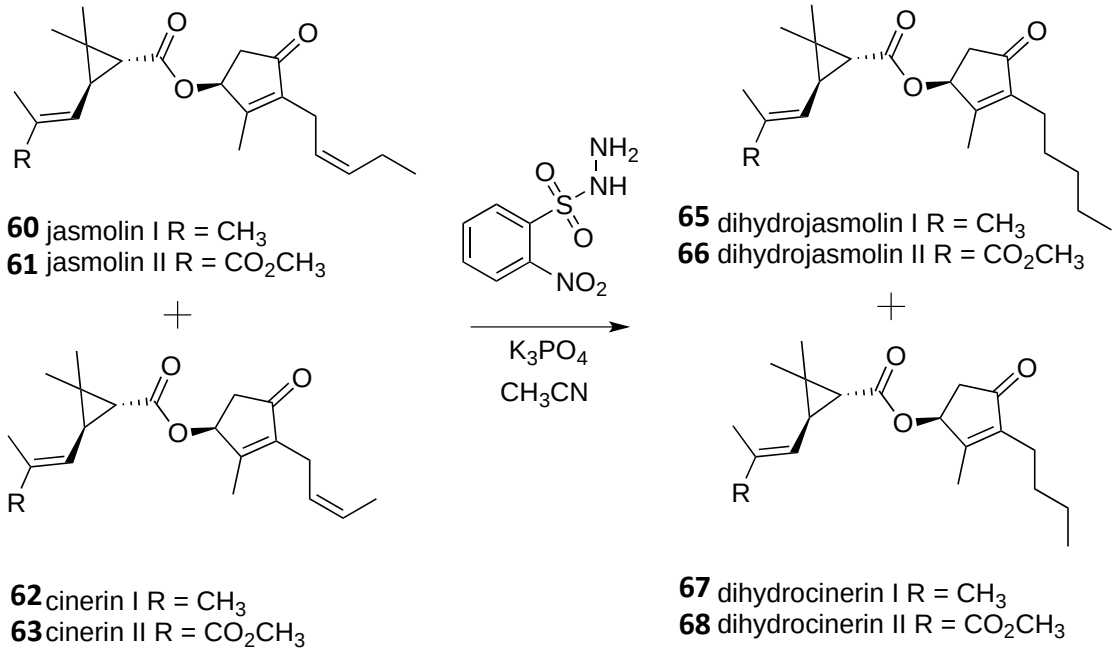


Figure 3.34 – GC chromatogram of diimide reaction Table 3.3, Entry 3.

From Entry 2 and Entry 3 in Table 3.3, it can be seen that the reaction of pyrethrin I and II takes around 42 hours to proceed to near completion. As discussed previously, diimide is not a long-lived molecule in solution, which suggests that the rate-limiting step in this process is the formation of diimide from NBSH. This study has shown proof of concept that the pyrethrin I and II may be hydrogenated with diimide with high selectivity, however, this is not a feasible process for industrial scale conversion. A more efficient method of generating diimide for this reaction would be the logical next step for this work. Myers and co-workers note that the formation of diimide from NBSH proceeds more rapidly in polar protic solvents such as water and methanol.[202] Therefore, a solvent screen for this reaction maybe appropriate.

The 'over-hydrogenated' dihydrojasmolin and dihydrocinerin products **65**–**68** have not been fully characterised in the literature. A protocol employing a high excess of NBSH over 72 h was undertaken so that the reaction would proceed completely to these compounds. The results from this experiment are shown in Table 3.3, Entry 4. This reaction was fractionated by flash chromatography to fractions containing the I series and II series of compounds. While the compounds were not isolated to purity and fully characterised as these derivatives have a similar polarity and co-elute in flash column chromatography, HRMS analysis of fractions containing these compounds provided strong evidence for their identity. In the ^{1}H NMR analysis, the lack of alkenyl signals is the key evidence for the formation of the fully saturated side chain. Further, the key signal indicating the presence of the cinerin components of the mixture is the small triplet at 1 ppm, which represents the terminal methyl group on the side chain of the molecule which is 1 carbon shorter in the cinerin derivatives **67** and **68** than in the jasmolin derivatives **65** and **66**.



Scheme 3.11 – Hydrogenation of jasmolin and cinerin to give dihydrojasmolin and dihydrocinerin

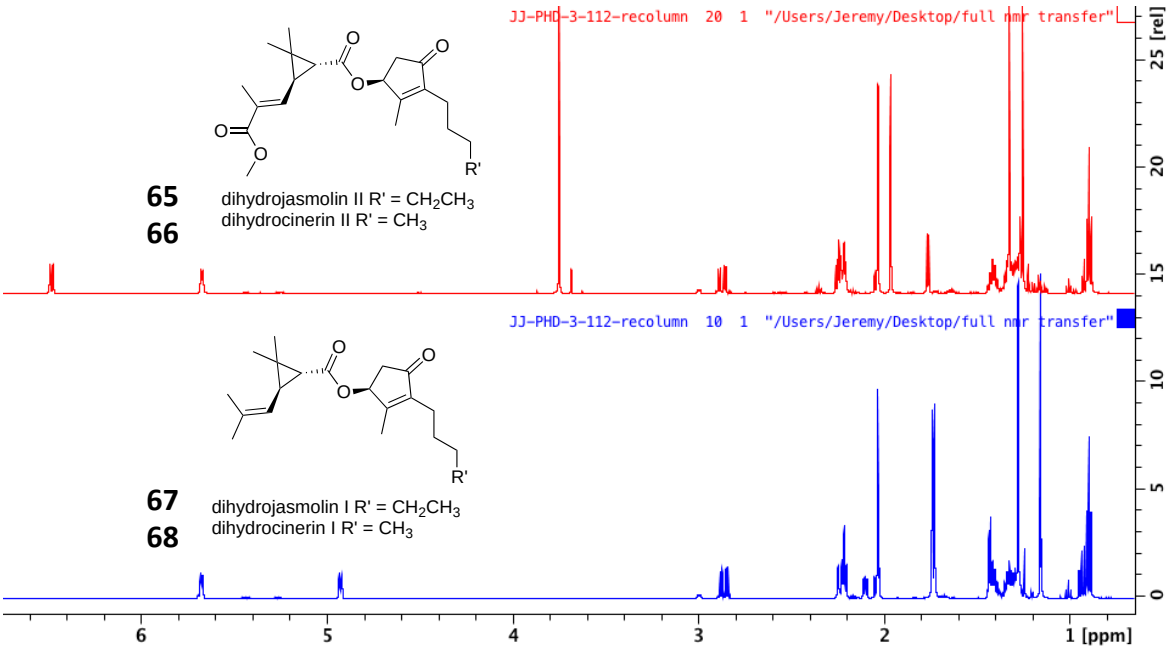
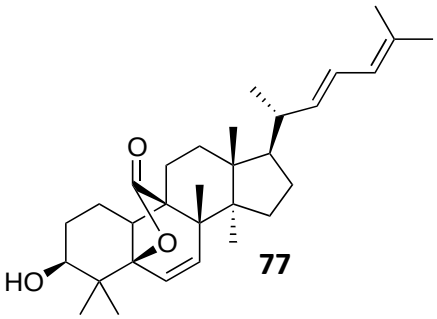


Figure 3.35 – ^{1}H NMR spectrum of the dihydrojasmolin I and dihydrocinerin I, bottom, and dihydrojasmolin II and dihydrocinerin II, top.

3.2.3 Crude Extract from Bitter Melon (*Momordia charantia*)

3.2.3.1 Background

Collaborators at the University of Tasmania's School of Medicine were interested in the studying biological properties of the extract from *M. charantia* (L.). Bitter melon is used frequently in Asian cooking, and has been used as a traditional medicine in Chinese and Indian culture.[206,207] Bitter melon has been shown to contain a wealth of biologically interesting molecules including cucurbitane triterpenoids such as **77**.[208-212] A wealth of other compounds have been isolated from this plant, reviewed in 2016.[213] The extract has shown a range of biological activities including anti-diabetic,[214] antioxidant,[215] and anti-cancer activity.[216,217]



77

Figure 3.36 – Cucurbitane type triterpene from *M. charantia*.

3.2.3.2 Extraction

Through the PHWE method, an extract was rapidly produced for testing. However, this particular plant provided some challenges in developing the method for extraction, particularly due to the significantly high water content of the material. Upon drying, the mass of material obtained indicated that the bitter melon fruits are approximately 92% w/w water. When exposed to the solvent inside the espresso machine, the plant material showed a high tendency to swell and cause blockages. Therefore, the method used for the preparation of the extract of bitter melon was 4 g of powdered bitter melon fruit, with 12 g of sand. The sample was extracted with 100% water, as a test extraction using and EtOH:H_2O mixture, followed by an extraction with an organic solvent did not yield any appreciable quantity of material.

Following the PHWE, the resulting aqueous extract was evaporated to dryness on the rotary evaporator and evaluated for biological activity without further purification. Ultimately, a multi-gram-scale bitter melon extract was produced in a yield of 25% w/w. The results of a subsequent study by collaborators into the anti-inflammatory activity of the bitter melon extract produced through PHWE are published.[218] In this study, the extract was tested against a human colonic adenocarcinoma cell line (LS174T). The extract showed no toxicity towards the cells, and showed a reduction in markers of endoplasmic reticulum (ER) stress. Therefore, this extract has the potential to be used as a treatment in inflammatory disorders such as inflammatory bowel disease (IBD). Future work in this project within the research group will be the isolation of compounds from the extract, in order to determine the bioactive metabolites.

3.2.4 Summary

Through the extraction of *E. adenophorum*, *C. cinerariaefolium* and *M. charantia*, the use of the PHWE method for the rapid generation of bioactive extracts was demonstrated. As for the extraction of *T. lanceolata* and *Correa spp.* (Chapter 1), the efficient extraction of somewhat sensitive substrates was achieved.

The extraction of sensitive terpene components from *E. adenophorum* convincingly demonstrated that this method is compatible with mildly air-sensitive compounds, provided that the resulting extract is treated appropriately. These compounds require minimal purification steps and handling time, therefore a rapid and simple purification is desired for optimal isolated yield. The combination of rapid PHWE extraction, and subsequent liquid-liquid extraction with heptane provided a crude extract highly enriched in the desired class of compounds leading to efficient purification, with each component requiring a maximum of two short flash chromatographic steps. These results also suggest that many of the compounds previously reported are artefacts of isolation through exposure to molecular oxygen by long extraction times and repeated chromatography.

Extraction of *C. cinerariaefolium* and subsequent liquid-liquid extraction with heptane rapidly provided an extract significantly enriched in pyrethrins. This extract showed very little evidence of other significant components aside from sesamin through GC-MS and NMR spectroscopic analysis, and was pale-green in colour. This suggested only trace

amounts of plant pigments and other coloured compounds, which is consistent with the other plants extracted. The ready ability to perform a selective diimide-mediated hydrogenation on the pyrethrin molecules within this extract further highlights the viability of this method for the rapid generation of enriched plant extracts.

3.3 Extraction of Known Valuable Natural Products

3.3.1 Marrubiin from White Horehound (*Marrubium vulgare*)

3.3.1.1 Background

M. vulgare (L.) is known to yield the compound marrubiin (**78**), which is known as a component of traditional medicine and shows analgesic properties. Studies into the structure of this compound were undertaken as early as the 1900's,[219,220] but the structure was not completely determined until 1953.[221] An X-ray study in 1983 provided conclusive evidence of the structure including stereochemistry of the product, which aligned with previous determination through synthetic studies.[222] Since then it has been extensively studied and shown that this compound and simple derivatives thereof have a number of different biological activities.[223-225] Numerous publications into the synthesis of this compound exist, including a total synthesis paper published in 2016.[226] This compound is not thought to be the true natural product, but an artefact of isolation. Premarrubiin (**78b**) is the true natural product which readily converts to marrubiin (**78**) on exposure to water and heat.[227]

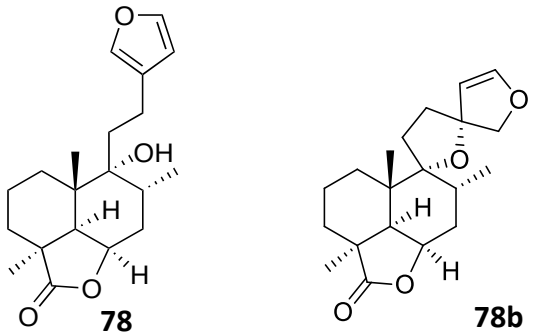


Figure 3.37 – Marrubiin (**78**) and premarrubiin (**78b**)

3.3.1.2 Extraction and Isolation

The extraction of this plant material with this PHWE method, and subsequent heptane extraction gave an extract enriched in the compound marrubiin (**78**). A fraction from the

chromatographic separation was taken which contained a high proportion of the compound, but was still not pure. However, trituration with hexane resulted in the isolation of marrubiin (**78**) as an amorphous solid. The spectral data obtained was consistent with that reported in the literature.[226]

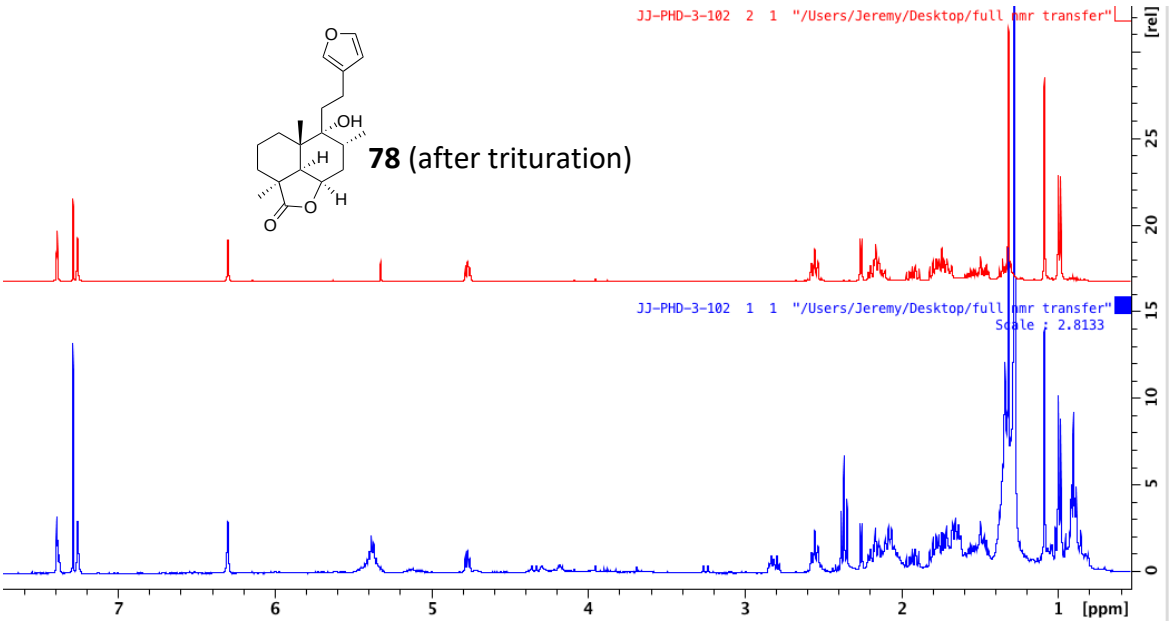


Figure 3.38 – ^{1}H NMR spectrum of the crude extract from *M. vulgare* (below) and the purified compound (above).

In spite of the low isolated yield (0.06 % w/w, much lower than a reported yield of 0.4 % w/w)[228], analysis of the ^{1}H NMR spectrum of the crude extract (862 mg from 15 g of *M. vulgare*, 5.75 % w/w) indicated a much higher content of **78**, which appears to decompose. This is demonstrated by significant reduction in the intensity of the furan resonances in the ^{1}H NMR spectrum at 6.27, 7.24 and 7.36 ppm (Figure 3.39). This reflects the importance of a method such as this novel PHWE method which is rapid, and has been shown to require less chromatography compared with other extraction methods for isolation of the compounds within the extract.

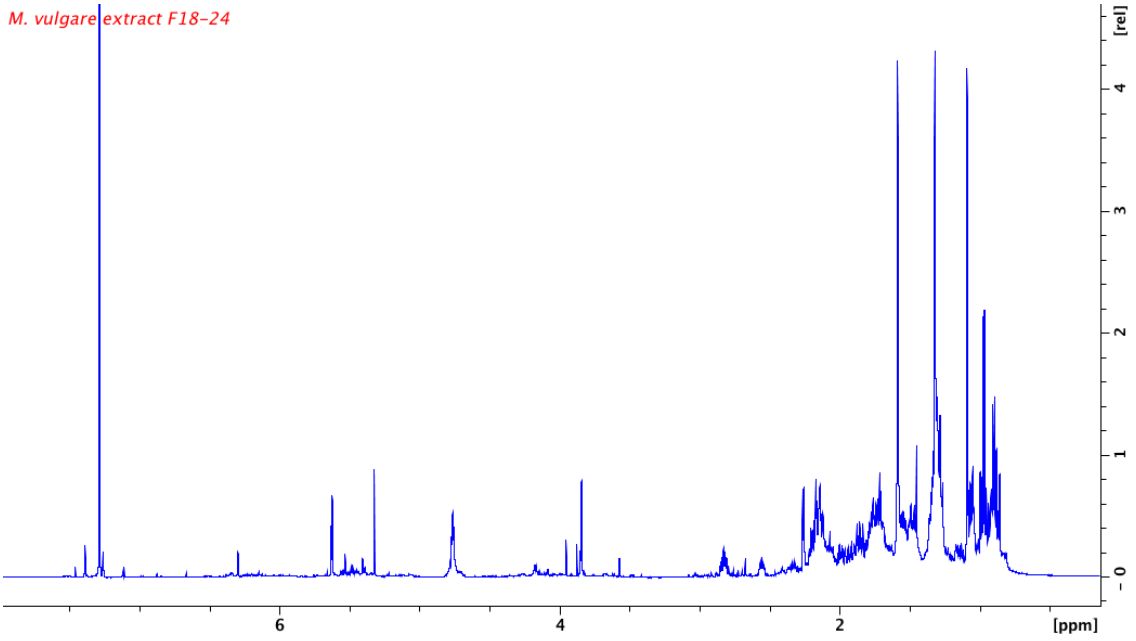


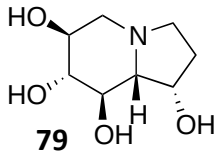
Figure 3.39 – ^{1}H NMR spectrum flash chromatographic fractions containing marrubiin, showing significant decomposition had occurred during purification.

In spite of these low yields, this material is of interest for synthetic applications within the research group, targeting natural products and analogues based on this scaffold. Commercial sourcing of the compound is not feasible, as with many of the examples detailed in this thesis.* Investigations are continuing into the improved isolation of this compound.

3.3.2 Castanospermine from Moreton Bay Chestnuts (*Castanospermum australe*)

3.3.2.1 Background

The toxic seeds of *C. australe* (A.Cunn & C.Fraser ex Hook.), also known as Moreton Bay Chestnuts, are known to contain the neurotoxic alkaloid castanospermine (**79**), which was first identified in 1981.[229] It has been extensively studied since then and shown diverse biological activity, including anti-HIV activity, anti-diabetic activity, and inhibition of various enzymes.[230-236]



79

Figure 3.40 – Castanospermine, isolated from *C. australe*

* ~USD$100 per 10 mg from Carbosynth, UK 14/11/17

The initial report details a procedure where 3 kg of the seeds are extracted with litres of 75% EtOH:H_2O, and separated by means of multiple ion exchange resin columns, and multiple crystallisation steps. The final yield of crystalline compound was 1.69 g, for an overall yield of 0.056% w/w. The X-ray analysis of the compound only gave relative stereochemistry, and the absolute stereochemistry was determined upon total synthesis of the compound from glucose, reported in 1984.[237]

Subsequently, the extraction and isolation of this compound was patented, with an improvement on the reported yield of 1.2 % w/w, representing the isolation of 3.5 g of crystals from 100 g of the fresh seeds. [238] A summary of the procedure from the patent is as follows:

> "(a) extracting the ground seeds with a mixture of water and 2-propanol;
>
> (b) washing the extract with petroleum ether;
>
> (c) chromatographing the solution on an acid resin with elution with ammonium hydroxide;
>
> (d) rechromatographing the eluate on a basic resin with elution with water; and
>
> (e) concentration of the aqueous eluate followed by trituration with acetone to give castanospermine."

Therefore, it was investigated if PHWE could be applied to this important compound.

3.3.2.2 Extraction and Isolation

For extraction of castanospermine (**79**) by espresso machine PHWE, the fresh seeds were grated before extraction of the wet material. It was observed that the seeds were unable to be extracted if allowed to dry out as they went very hard and could not be ground up. Amberlyst® 15 acidic ion exchange resin was added to bind to the castanospermine, and the supernatant was then filtered away from the resin. The resin was then eluted with a methanol:ammonia solution, to provide an extract enriched in castanospermine. Purification by automated gradient flash chromatography yielded the compound as a white amorphous solid, in a yield of 0.56 % w/w (565 mg). This is 10x higher than the initially reported yield of the compound but around half that achieved and reported in

the patent, though in much less time (which may be due to variation of concentration of the compound within the seeds).[229,238] This is another valuable natural product, and therefore this represents another example of viable extraction of compounds that are expensive to source commercially. *

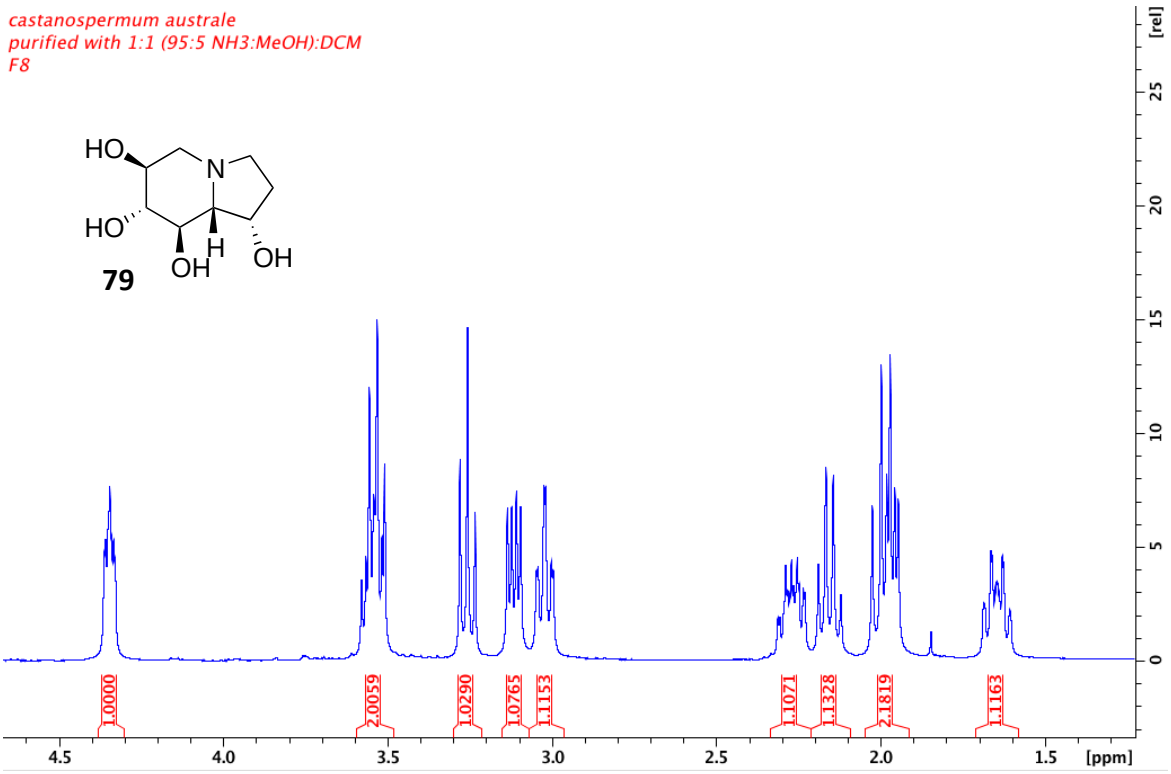


Figure 3.41 – ^{1}H NMR spectrum of castanospermine in D_2O.

3.3.3 Summary

The extraction of marrubiin (**78**) and castanospermine (**79**) demonstrate the application of the method for rapid extraction of known valuable natural products which may be used as starting materials for synthesis. The extraction of marrubiin from *M. vulgare* showed that in spite of the sensitive nature of the target compound, the PHWE extract contained a high proportion of this valuable natural product. Purification of this compound proved problematic, with significant decomposition observed, highlighting the viability of the extraction method for extraction of sensitive compounds.

The extraction of castanospermine from *C. australe* provided quantities of this material sufficient for small-scale synthetic studies. However, this method may not be as efficient as other methods for extracting this compound, particularly on a larger scale. This further

* AUD$281 for 10 mg of material from Sigma–Aldrich, 01/11/17

demonstrated that traditional natural product extraction techniques are still important for various plants, and that the PHWE method is a complementary extraction technique that like all extraction methods has its limitations.

3.4 Bioprospecting/Chemotaxonomic Applications

3.4.1 Drimanes from Wielangta Forest Tasmanian Native Pepper (*Tasmannia lanceolata*)

3.4.1.1 Background

A specimen of *T. lanceolata* from Wielangta Forest in Tasmania which contained a previously unreported derivative of polygodial (**12**), 1β-acetoxypolygodial[105] (**80**) as well as the known lower oxidation state 1β-acetoxyisodrimeninol (**81**), was discovered and reported by the Smith group.[105]

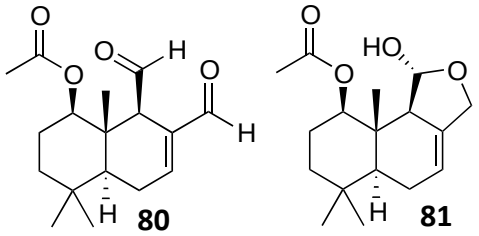


Figure 3.42 – 1β-acetoxypolygodial (**80**) and 1β-acetoxyisodrimeninol (**81**).

This was a serendipitous discovery during an undergraduate field-trip to collect samples for natural product laboratory classes in 2012. All previously investigated *T. lanceolata* samples including those received from Essential Oils of Tasmania and Diemen Pepper showed no evidence of containing 1β-acetoxypolygodial. In this original report, traditional extraction methods were used. The purification of **80** required multiple chromatographic separations, and the isolated crystals were still green due to the presence of plant-based chlorophylls.[105] Based on this different metabolite, it was hypothesised that this specimen may be a new genetically different subspecies of *T. lanceolata*. Therefore, to investigate this further, samples of *T. lanceolata* from the surrounding area were collected to demonstrate the viability of this new PHWE method for a rapid pilot chemotaxonomic study of Wielangta Forest *T. lanceolata* specimens. Initially, it was hypothesised that there would be trees which exclusively contained either polygodial (**12**), or 1β-acetoxypolygodial (**80**).

3.4.1.2 Initial Extraction

A second sample from the original specimen was extracted, and following subsequent liquid-liquid extraction with heptane, the following ^{1}H NMR spectrum was obtained.

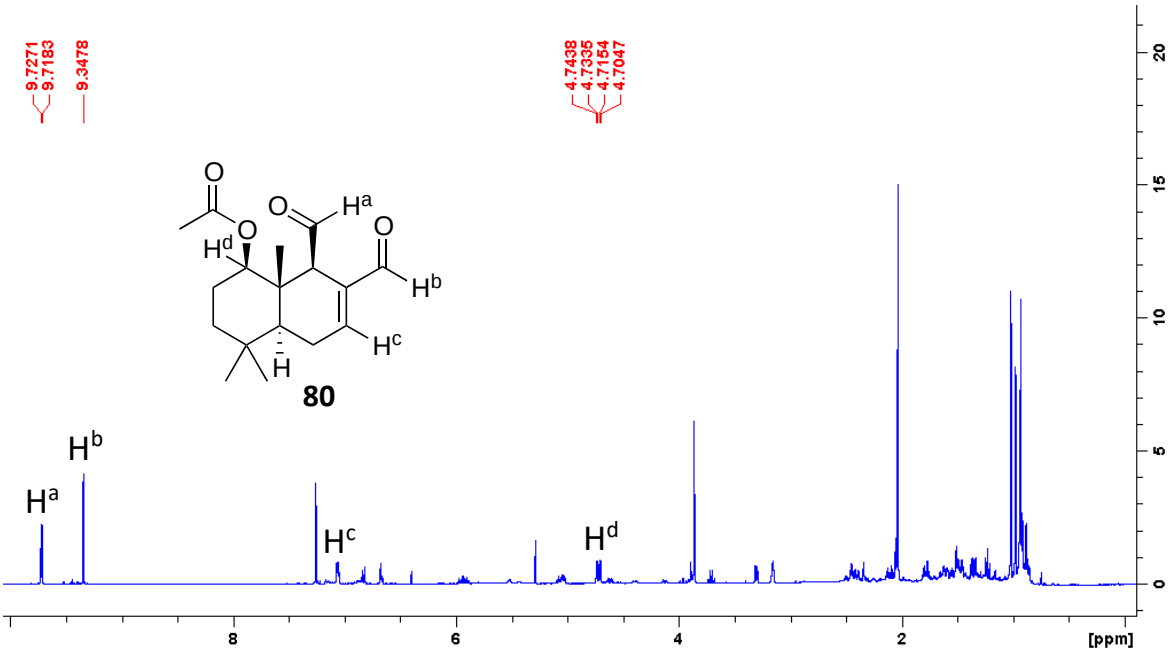


Figure 3.43 – ^{1}H NMR spectrum of the extract from Wielangta forest *T. lanceolata* containing 1β-acetoxypolygodial (**80**).

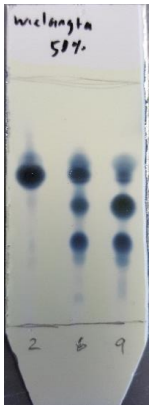
Key signals in the ^{1}H NMR spectrum are the aldehyde signals at 9.72 and 9.35, which differ significantly in chemical shift from the aldehyde signals for polygodial (**12**), and the doublet of doublets at 4.72, which represents the proton attached to the carbon at the C-1 position where the acetoxy functionality is attached.

3.4.1.3 Survey of a Population of *T. lanceolata* from Wielangta Forest

In 2017, a further ten samples of *T. lanceolata* were taken from the surrounding area in Wielangta Forest. GPS locations for all the trees that were sampled in this experiment were taken, and are shown on the following map (Figure 3.44). This sample of trees covered only a very small area of the forest, but was deemed sufficient for a preliminary chemotaxonomic survey.

Figure 3.44 – Map showing the locations of the *T. lanceolata* specimens sampled in the survey.

Each sample was extracted and the ratio of the products was determined by ^{1}H NMR spectroscopic analysis. Each sample was also purified to isolate the individual components. The initial results showed that each sample contained a varying amount of each substance. This result was contrary to the original hypothesis that each sample may contain either polygodial (**12**) or 1β-acetoxypolygodial (**80**), but not both. Additionally, all trees that contained 1β-acetoxypolygodial, also contained the corresponding hemiacetal derivative 1β-acetoxyisodrimeninol (**81**) which is consistent with the original sample. Some samples contained a majority of one or the other compound, and others had a more even mixture. Compounds **12**, **80** and **81** showed good separation by TLC analysis as shown in Figure 3.45, and baseline separation by automated gradient flash column chromatography as shown in Figure 3.46.



12 Polygodial
80 1β-acetoxypolygodial
81 1β-acetoxyisodrimeninol

Figure 3.45 – TLC analysis of Wielangta Forest *T. lanceolata* samples 2, 6 and 9.

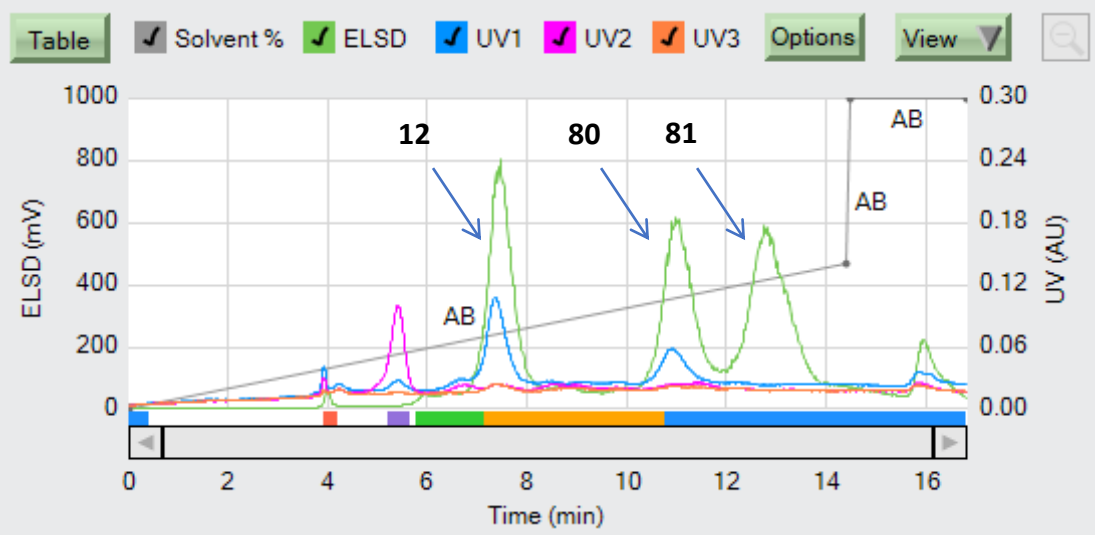


Figure 3.46 – Chromatogram of the separation of *T. lanceolata* sample 6, showing separation of the 3 major components.

Table 3.4 and Figure 3.47 summarise the results. Notably, Sample 2 showed polygodial (**12**) as overwhelmingly the major component with none of the 1β-acetoxy derivatives (**80** + **81**) observed, and sample 9 showed the major components being 1β-acetoxy derivative (**80**). Other samples showed a much more balanced level of the major components. The yield of each of the compounds within the plant varied widely (~0.1 % – ~1.4 % for **12**, 0 % – ~1.2 % for **80**, and 0 % – ~1.0 % for **81**), as did the ratio between them, as well as the overall yield of drimanes from each sample (0.48–2.93 % w/w). There also did not appear to be any correlation between the relative levels of the 1β-acetoxy derivatives (**80** + **81**). This survey was not of a sample size large enough to draw definitive conclusions about the distribution of specimens vs. the compounds isolated.

However, these results did indicate that the population of trees surveyed in Wielangta Forest contained varying amounts of these compounds, and did not exclusively contain 1β-acetoxy derivatives **80** and **81** as originally hypothesised.

Sample	Plant mass (g, dry)	**12** mass, g	**80** mass, g	**81** mass, g	Total yield, g, (% w/w)
1	60.0	0.113	0.405	0.266	0.784 (1.31 %)
2	31.5	0.459	0.000	0.000	0.459 (1.46 %)
3	44.1	0.050	0.207	0.266	0.523 (1.19 %)
4	6.8	0.032	0.013	0.072	0.117 (1.72 %)
5	47.1	0.071	0.106	0.058	0.235 (0.50 %)
6	31.2	0.079	0.075	0.090	0.244 (0.78 %)
7	25.7	0.111	0.193	0.104	0.408 (1.59 %)
8	40.0	0.056	0.142	0.118	0.316 (0.79 %)
9	32.6	0.034	0.111	0.012	0.157 (0.48 %)
10	32.0	0.198	0.404	0.336	0.938 (2.93 %)
Total g, % w/w	351.0	1.203 g, 0.34 %	1.656 g, 0.47 %	1.200 g, 0.34 %	4.059 g, 1.15 %

Table 3.4 – Summary of the extraction yields from each of the samples, and the total amount of each compound obtained.

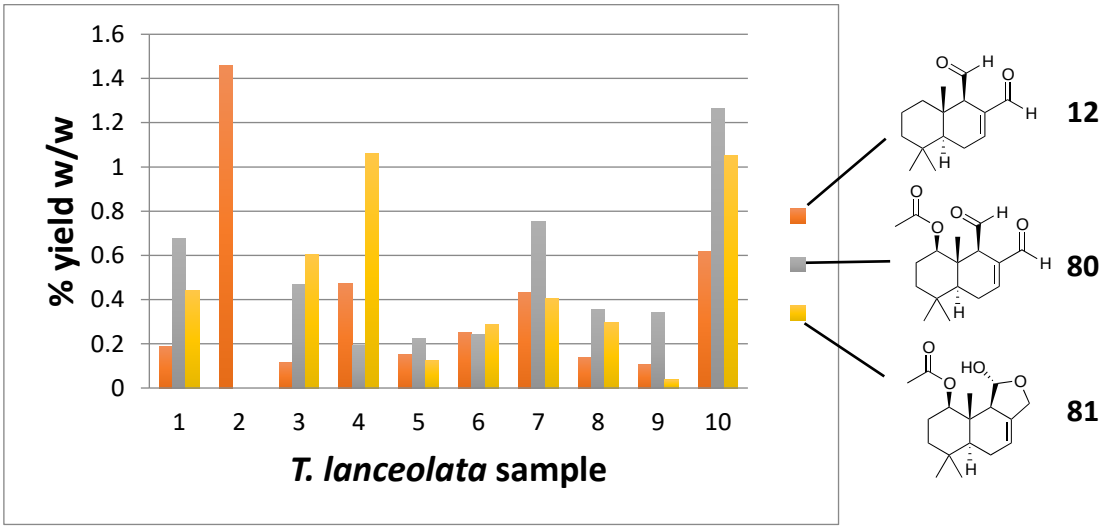


Figure 3.47 – Yield of compounds isolated from Wielangta Forest *T. lanceolata.*

Overall, 1.656 g of 1β-acetoxypolygodial (**80**), 1.200 g of 1β-acetoxyisodrimeninol (**81**), and 1.203 g of polygodial (**12**) were isolated, representing an overall yield of 0.47 %, 0.34 %, and 0.34 % w/w respectively, and therefore a total overall yield of 1.15 % w/w of total drimanes from all the samples combined. Typically, these plant samples may be

combined and extracted as a bulk homogenous sample. However, the rapid nature of the PHWE extraction method allowed the efficient extraction of each sample individually to build a chemotaxonomic profile of the area surveyed.

It is not currently understood why these specific trees produce 1β-acetoxypolygodial (**80**) when it has never been reported previously in the literature, and has only been found in one location in Tasmania despite the extensive literature on this species. In addition, no conclusions were made as to why the level and ratio of the compounds varies to such an extent in this population of trees. Secondary metabolites may vary within individuals of a species for a variety of complex reasons, highlighted in a 2014 review by Moore and co-workers.[238a] Further investigations would be necessary to determine the reason for this unusual metabolite occurring in this population of specimens.

3.4.2 Extraction of Winter's Bark (*Drimys winteri*)

3.4.2.1 Background

Drimys winteri (J.R. Forst. & G. Forst.) is related to *T. lanceolata*, and is known to contain polygodial (**12**).[239] This plant was extracted to investigate polygodial content, as well as to identify other major components. *D. winteri* has also been previously found to contain 9-epipolygodial (**14**) and (–)-drimenol (**118**).[240,241] Flavonoid compounds taxifolin and astilbin (**82**) have also been isolated from the bark of this plant.[239]

3.4.2.2 Extraction and Isolation

The leaves were extracted using the PHWE method, and the resulting extract fractionated through extraction with heptane, followed by EtOAc. The residual aqueous extract was subsequently evaporated to dryness, and the ^{1}H NMR spectra of these extracts showed a high degree of selectivity in the of fractionation of components, as shown above for *E. adenophorum*.

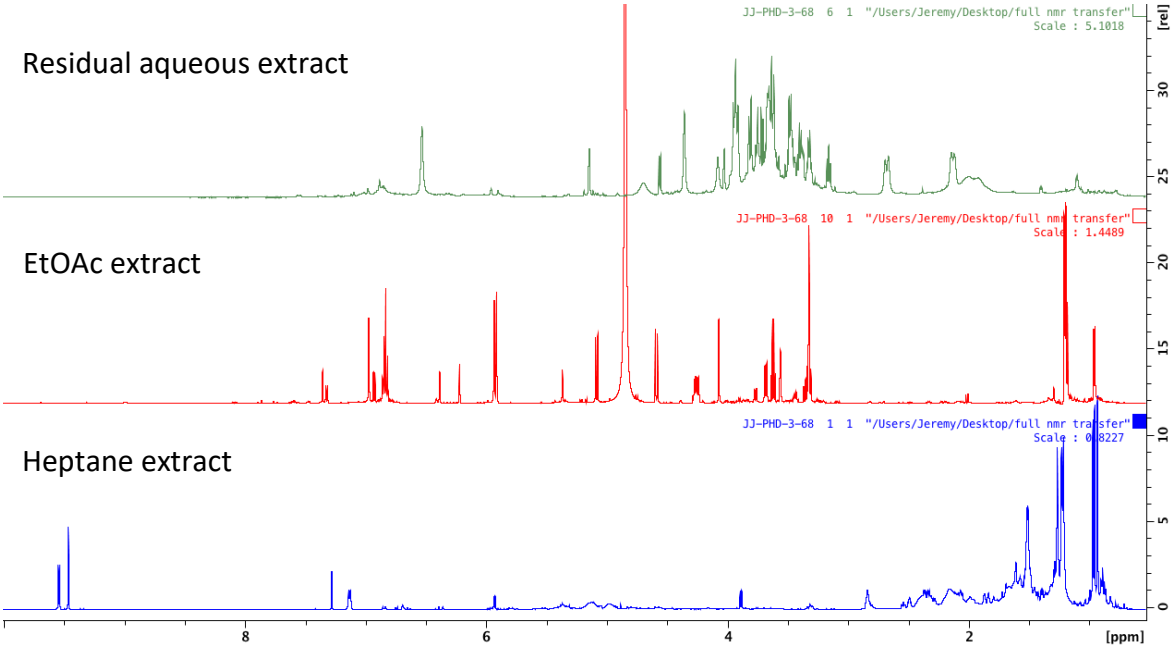


Figure 3.48 – ^{1}H NMR spectra of *Drimys winteri* fractions (from top) aqueous extract in D_2O, EtOAc extract in MeOD, heptane extract in $CDCl_3$.

The ^{1}H NMR spectrum of the heptane extract clearly showed polygodial (**12**) as the major component, with the diagnostic aldehyde signals at 9.53 and 9.46 ppm, the alkene at 7.12 ppm, and the three characteristic methyl singlets of the drimane skeleton around 1 ppm. The mass of the crude heptane had a yield of 2.1 % w/w, indicating that this sample of *D. winteri* does not contain a level of polygodial equivalent to the high content *T. lanceolata*, so extraction of this plant for the polygodial content was not pursued. The EtOAc extract, however, had a much higher crude mass, with a crude yield of 12.1 % w/w. In addition, ^{1}H NMR spectroscopy and TLC analysis indicated two major components. These compounds proved difficult to separate by normal phase column chromatography, with co-elution yielding 1.44 g (8.4 % w/w) of a fine yellow powder. These compounds were readily separable in reversed-phase mode, and after isolation were identified as astilbin (**82**) and quercitrin (**83**) by comparison with NMR data with that previously reported.[242,243] These two compounds differ only in the degree of saturation in the heterocyclic ring. Only a small sample of the two compounds were separated into their individual components due to the size of the reversed-phase column used, but the ratio of the compounds gives the overall yield of the two compounds. The ^{1}H NMR spectrum shows a ratio astilbin:quercitrin 1:0.44, which gives approximate yields

of these compounds of 5.8 % and 2.6 % w/w respectively, indicating that these compounds may be readily obtained in high yields from this plant.

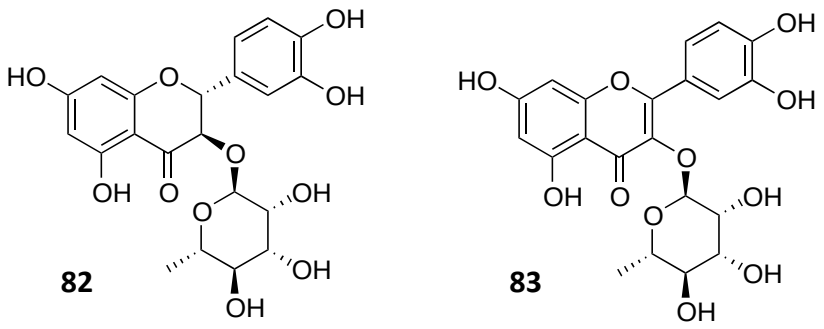


Figure 3.49 – Astilbin and quercitrin isolated from *D. winteri*

Astilbin has been previously isolated from *D. winteri* in a 0.20 % yield w/w.[239] Quercitrin has been isolated from the closely related *D. brasiliensis*.[89] These compounds have known biological activity, being actively studied in 2017,[244-247] and are relatively expensive to source commercially.*

3.4.3 Summary

The extraction of the *T. lanceolata* from Wielangta Forest demonstrates the utility of the method for rapid extraction of individual samples for rapid bioprospecting and chemotaxonomic analysis. The extraction of multiple samples for comparison of the relative levels of major components was simplified as the enrichment of the extract allowed baseline separation of the major components in a single flash chromatographic run, and therefore an accurate quantitative assessment of each sample. Not only did this method provide a rapid method for comparison, but also allowed isolation of gram-scale quantities of the three major components.

The extraction *D. winteri* again demonstrates the flexibility of the method for the extraction of compounds of a wide range of polarity, which in turn further highlights the validity of PHWE as a bioprospecting method. The initial target from extraction of this plant was the non-polar terpene polygodial. However, the most significant result was the very high quantities of valuable glycosides, which were obtained during the extraction.

* Astilbin – USD$252 for 25 mg from AK Scientific, Quercitrin – USD$130 for 20 mg from Arctom Chemicals USA 14/11/17

The significantly higher yield of astilbin from *D. winteri* also demonstrates the advantage of the PHWE method, where a single chromatographic step is required for isolation of components, which leads to less losses through sample handling and decomposition.

3.5 Extraction of Essential Oils from Common Culinary Plants

3.5.1 Overview

Due to the utilitarian nature of this method, it was anticipated that it could be readily integrated into the undergraduate laboratory program at the University of Tasmania as a replacement/alternative to traditional hydrodistillation. To this end, a number of common culinary plants were extracted, targeting the essential oil components. Combined, these demonstrate the ease of generation of extracts enriched in essential oils. Cloves, star anise, caraway seeds, thyme, oregano and lemon myrtle were all extracted to provide extracts enriched in the major components.

3.5.2 PHWE in the Undergraduate Laboratory

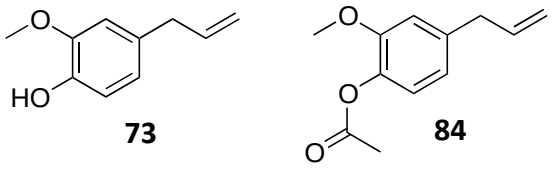
Natural Products extraction in the undergraduate teaching laboratory often involves hydrodistillation of essential oils from plants. Indeed, as recently as 2015, undergraduate experiments pertaining to the extraction of monoterpenoid natural products from common culinary plants using this method have been reported.[248] As discussed through this thesis, PHWE is an increasingly popular method of extracting organic components from various complex matrices including plant materials. Providing students with hands-on experience at an undergraduate level, however, can be expensive/impractical due to the costly nature of the equipment involved. To that end, the espresso machine PHWE has been incorporated into the undergraduate laboratory at second- and third-year levels as an inexpensive option to custom-made instruments.[75]

Implementation of this work into the undergraduate laboratories serves many purposes. In incorporating work from research groups within the building into the curriculum, the students are able to more clearly see the applications of the topics taught during their undergraduate education. Additionally, this experiment replaced a traditional hydrodistillation, so the students are exposed to a broader range of techniques. The hydrodistillation required an expensive set of glassware for every student, and the distillation takes a substantial amount of the laboratory time to complete. In contrast, two espresso machines are sufficient for a laboratory of ~16 students, who can quickly complete the extractions one after another and therefore accomplish more within the laboratory session.

This section describes the extraction and isolation of major components from common culinary plants. Such plants are often used, due to being available, and the simplicity of the resulting extraction and analysis of the major components.[249] The degree of enrichment of the ^{1}H NMR spectra for the resulting extracts for these plants makes these excellent examples for undergraduate natural products chemistry.

3.5.2 Cloves (*Syzygium aromaticum*)

The hydrodistillation of eugenol (**73**) and acetyleugenol (**84**) from cloves, the flower buds of *S. aromaticum* ((L.) Merrill & Perry), was an experiment already performed by second year undergraduate students at the University of Tasmania up until 2014. Investigations into the efficacy of the PHWE method for the extraction of these compounds were undertaken. This method was subsequently incorporated into the undergraduate laboratories and supplanted the traditional steam distillation experiment.



73 **84**

Figure 3.50 – Eugenol and acetyleugenol

Espresso machine PHWE extraction with 25% EtOH:H_2O (200 mL) provided a brown crude extract. CH_2Cl_2 has been typically used as the extraction solvent but limiting the use of toxic halogenated solvents by undergraduate students is desirable. Therefore, heptane was trialled as an alternative solvent. This is preferable to the more common hydrocarbon solvent, hexane, which is a potential neurotoxin.[250-252] To compare the extraction yield for these two solvents, cloves (15 g) were ground and extracted by PHWE. The resulting extract was extracted with heptane to generate a crude essential oil extract. Following acid–base extraction to separate the acidic eugenol (**73**) from the non-acidic acetyleugenol (**84**) The resulting extract containing eugenol (**73**) was split into two portions before one fraction was extracted with heptane and the other with CH_2Cl_2. Upon isolation of the product, the respective masses of the resulting pale-yellow oils were 636 mg and 646 mg which were shown by GC-MS and ^{1}H NMR spectroscopic analysis to be one major component, providing evidence that heptane is an efficient solvent for the extraction of eugenol from an aqueous mixture. The overall yield of the

extraction was 8.5 % w/w. Acetyleugenol (1.2% w/w) was the primary component of the neutral fraction. These results are comparable with traditional hydrodistillation, which demonstrates that PHWE is a viable alternative, and offers the benefit of a more rapid extraction, and low cost per student to implement into the laboratories.

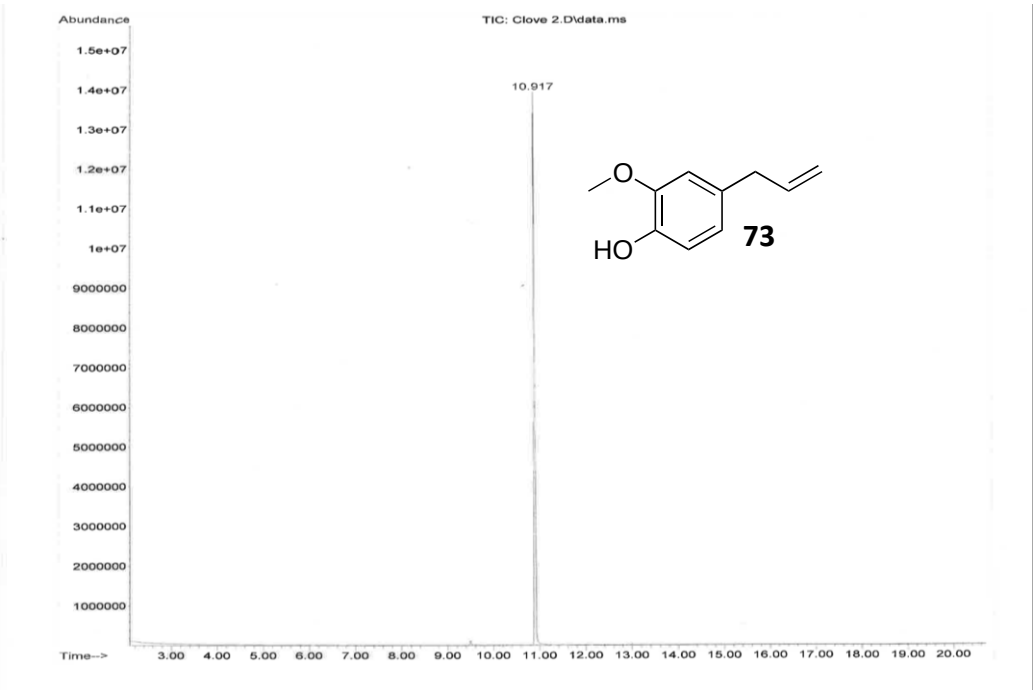


Figure 3.51 – GC analysis of the resulting eugenol from PHWE and acid–base extraction.

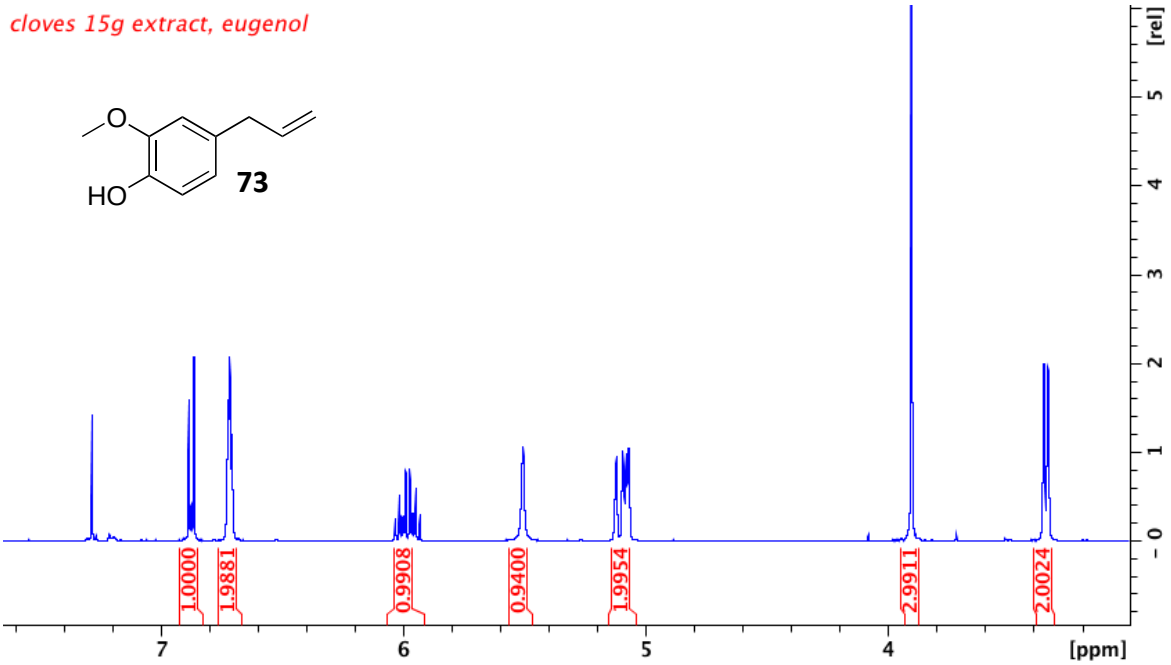
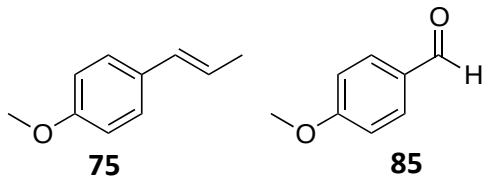


Figure 3.52 – ^{1}H NMR spectrum of the resulting eugenol from PHWE and acid–base extraction.

3.5.3 Chinese star anise (*Illicium verum*)

Aside from being a rich source of shikimic acid (see Chapter 2), star anise also contains a large essential oil fraction, with two major components, anethole (**75**) and *p*-anisaldehyde (**85**). Ground star anise seeds and seed pods were extracted by PHWE (25% EtOH:H_2O), followed by extraction with CH_2Cl_2 provided a crude green extract (3.8 % yield w/w). The resulting pale yellow/green oil showed the two major components identifiable in the ^1H NMR spectrum, in a ratio of approximately 13:1 anethole:*p*-anisaldehyde **75**:**85**.



75 **85**

Figure 3.53 – Anethole and p-anisaldehyde

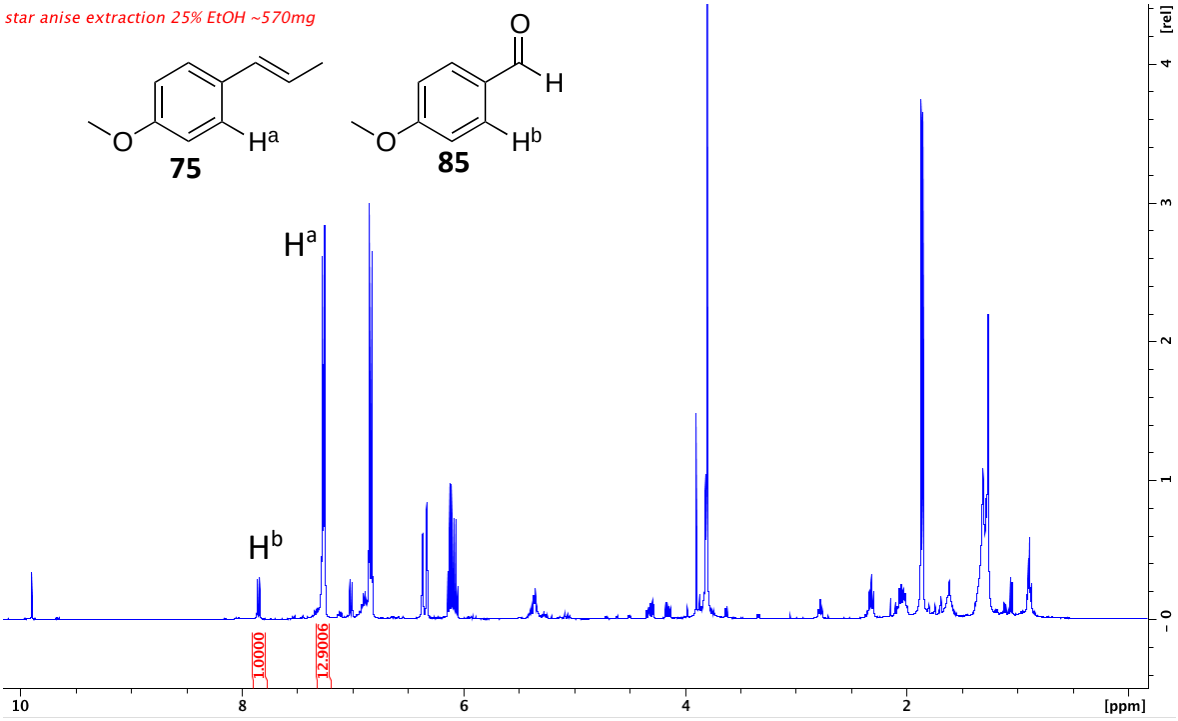
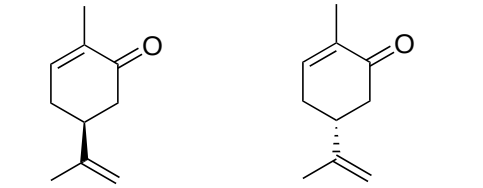


Figure 3.54 – ^1H NMR spectrum of the CH_2Cl_2 extract from Chinese star anise showing the major components and their ratios.

3.5.4 Caraway seeds (*Carum carvi*)

Caraway seeds (*Carum carvi* L.) contain the fragrant flavour compound *S*-(+)-carvone (**86**), which is enantiomeric with *R*-(–)-carvone which is the spearmint flavour from the spearmint plant (*Mentha spicata*). Ground caraway seeds were extracted by PHWE (30%

EtOH:H_2O), followed by extraction with CH_2Cl_2. The resulting yellow oil (127 mg, 0.85 % w/w from 15.0 g of seeds) showed clearly the major component in the ^1H NMR spectrum. The difference in smell of these enantiomers is a classic demonstration of the importance of stereochemistry, so extraction of spearmint could be undertaken to obtain the enantiomer.



(S)-(+)-carvone **86** (R)-(–)-carvone **87**

Figure 3.55 - Enantiomers of carvone.

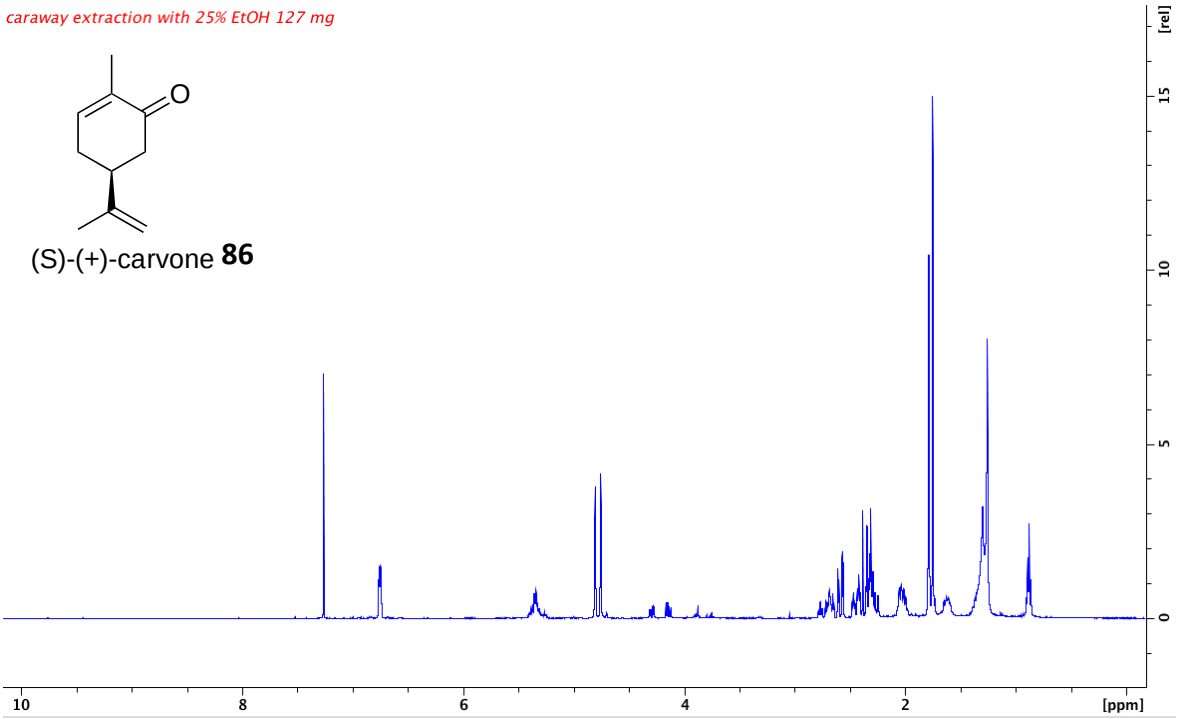
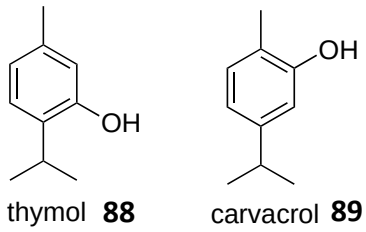


Figure 3.56 – ^1H NMR spectrum of the CH_2Cl_2 extract from caraway seeds.

3.5.5 Oregano (*Origanum vulgare*)

Oregano (*Origanum vulgare* L.) contains a complex mixture of compounds in its essential oil fraction. The major components are regioisomers thymol (**88**) and carvacrol (**89**). These compounds can exist in varied ratios in the plant.[253] Two different brands of oregano (15.0 g and 12.5 g) were extracted by PHWE (25% or 30% EtOH:H_2O), which had

very different ^{1}H NMR spectral profiles. The yields for these extractions were 1.46 % (204 mg) and 0.41 % (61 mg) (w/w) respectively.



thymol **88** carvacrol **89**

Figure 3.57 - Regioisomers thymol and carvacrol

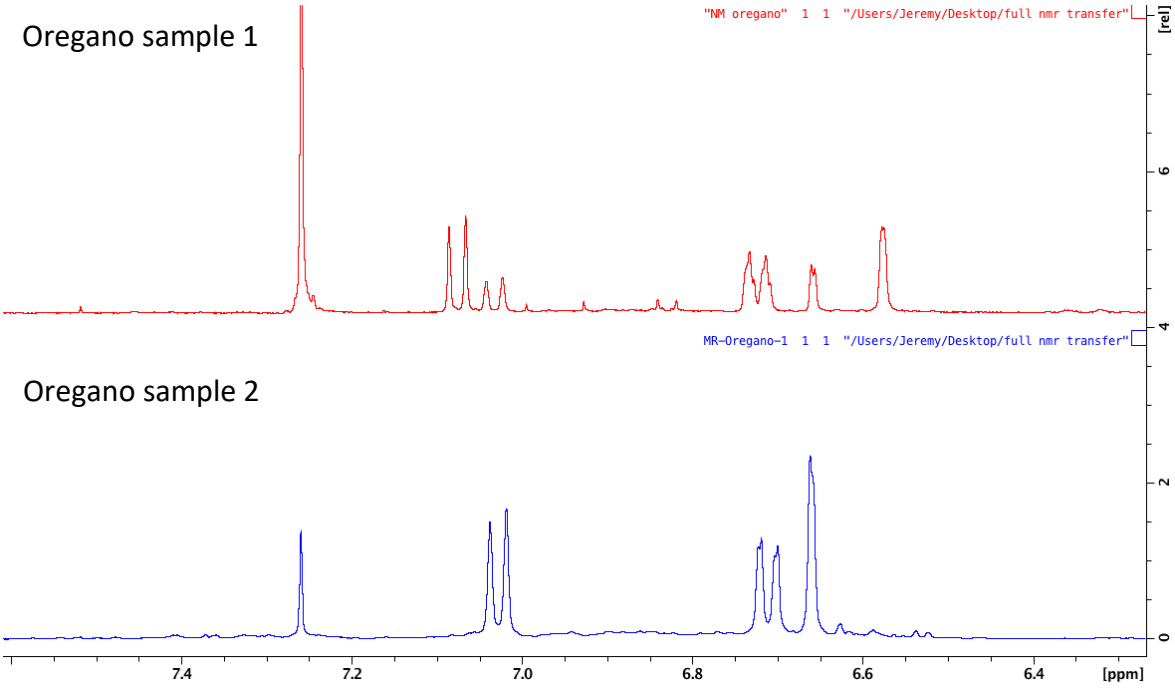


Figure 3.58 – Partial ^{1}H NMR spectra of the two different oregano extracts showing a difference in their chemical composition.

It can be seen that the bottom NMR spectrum (zoomed in on the aromatic region) shows 1 major compound, and the top NMR spectrum shows a mixture of compounds. The bottom fraction contains only carvacrol (**89**), whereas the top spectrum contains thymol (**88**) and carvacrol (**89**) in a ratio of ~2:1. This example further highlights how this method could be applied as an analytical tool due to the rapid preparation of the extracts, as was demonstrated for 1β-acetoxypolygodial (**80**) previously (Section 3.4.1).

3.5.6 Thyme (*Thymus vulgaris*)

The essential oil of thyme (*Thymus vulgaris* L.) also contains thymol as a major component, but lacks the carvacrol that oregano contains.[254] Extraction of ground thyme leaves by PHWE (30% EtOH:H_2O), followed by extraction with CH_2Cl_2 provided a crude green extract (1.46 % yield w/w). This extract was primarily composed of thymol (**88**), determined by analysis of the ^{1}H NMR of the crude extract.

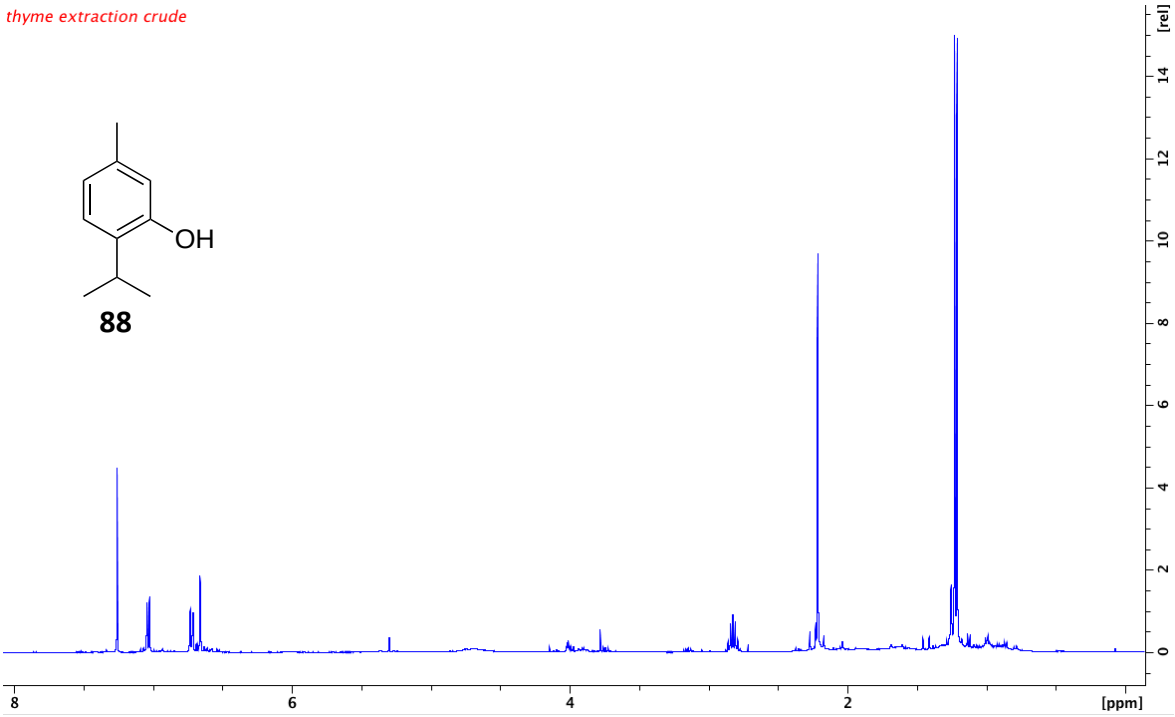


Figure 3.59 – ^{1}H NMR spectrum of the CH_2Cl_2 extract of thyme

3.5.7 Lemon Myrtle (*Backhousia citriodora*)

Lemon myrtle (*Backhousia citriodora* F.Muell.) is known to contain a mixture of compounds known as citral (**90** + **91**). Citral is a mixture of double bond isomers, geranial (**90**) and neral (**91**), which possess a strong but pleasant citrusy odour. These compounds make excellent candidates for study by undergraduate students as they are highly abundant in readily available plants, and can undergo simple chemoselective reactions due to the different functional groups they possess.[255] The two compounds are difficult to separate by standard flash column chromatography but they can be selectively hydrogenated to form a single compound, citronellal.[255]

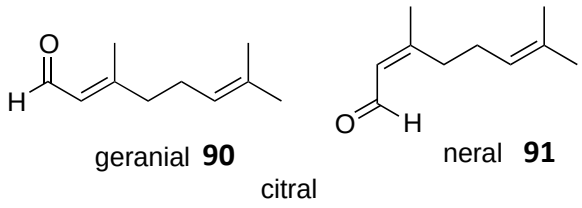


Figure 3.60 – Stereoisomeric components of citral

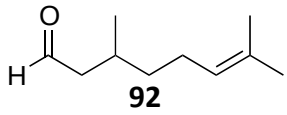


Figure 3.61 - Citronellal

Dried lemon myrtle (*Backhousia citriodora*) leaves were ground and extracted using the PHWE method (35% EtOH:H_2O). Subsequent extraction with CH_2Cl_2 provided a pale yellow oil in a yield of 8.1% w/w. The resulting ^1H NMR spectrum showed an extract that is relatively clean and contains primarily **90** and **91**. The most distinct peaks are those of the aldehydes, which can be used directly on the crude extract to determine the ratio of geranial to neral in the extract. The observed ratio of 2:1 is consistent with previous findings.[256] This extract can be used for subsequent chemical modification without purification due to the lack of significant impurities.

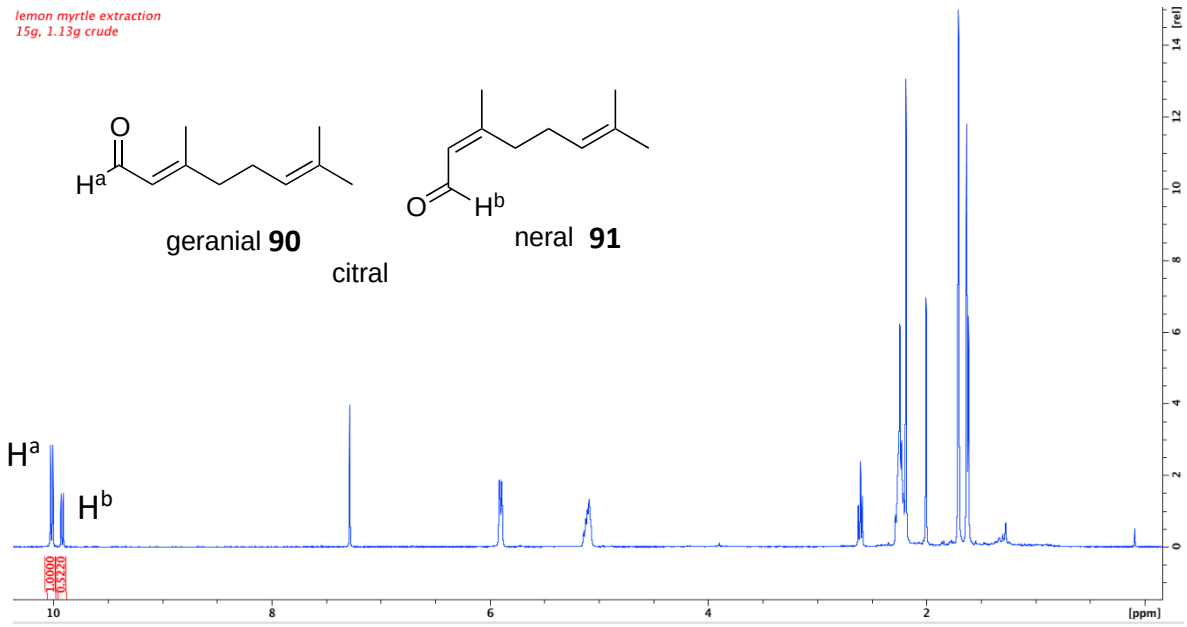


Figure 3.60 – ^1H NMR spectrum of the crude extract CH_2Cl_2 extract of lemon myrtle, showing the ratio of the two aldehydes present.

3.5.8 Summary

These extractions have demonstrated the ease with which extracts enriched in essential oils may be obtained through the PHWE method. This showed that the PHWE method may be used as an alternative to hydrodistillation, further highlighting the flexibility and application of this method to a broad range of substrates.

The implementation of the method in the undergraduate laboratories also represents a significant achievement. Substituting the PHWE technique removed a distillation, which was a previously learned technique for most of the students, and this new method allows students to be exposed to current research developments. Extracts such as those considered make excellent teaching examples for students, as they can see major components in the ^{1}H NMR spectra of the crude material. Further, by adding TLC analysis and chromatography, developing undergraduate experiments at varying levels is possible. Student feedback is generally positive about the use of this method, with many expressing surprise that the method is so effective, and commenting that it is a unique and interesting idea which hopefully will aid in students thinking outside the box in future academic pursuits.

3.6 Subsequent Espresso Machine PHWE Applications

Other members of the research group at the University of Tasmania have undertaken further research exploiting the rapid and efficient nature of this novel method for extraction of interesting molecules. This includes large scale extraction of the important bioactive molecule asperuloside,[257] and the discovery of new diterpene carboxylic acid natural products from *Dodonaea viscosa*.[88] A significant synthesis project was also undertaken on the synthesis of pyrrolic derivatives of polygodial targeting other families of natural products.[258] There are other current projects underway in the research group, focussing on the extraction of metabolites from endemic Tasmanian plant species for bioprospecting and chemotaxonomic applications.

National and international collaborations have been established through this work, with gram–scale quantities of polygodial sent to the USA, as well as Belgium. The research groups in these countries had a need for this compound for their research but were unable to source it efficiently.

Since this research was undertaken, another research group has explored espresso machine extraction. A research group in Spain are employing a different type of espresso machine for their own research.[259-262] The group uses a hard cap espresso machine, more commonly known as a 'pod' espresso machine, so named because instead of using a sample basket for the plant material, the material is packed into disposable pods. This research group are using these machines for analytical applications, which shows another application not currently exploited by the research group at the University of Tasmania. The ability to use an espresso machine for quantitative extraction as required for analytical applications, further highlights the viability of espresso machines as high-performance laboratory equipment.

3.7 Conclusion

The examples provided highlight the significance and scope of the espresso machine PHWE method. The rapid extraction of bioactive extracts such as the pyrethrins from *C. cinerariaefolium* and terpenoids from *E. adenophorum* has provided further collaboration for the research group and therefore expansion of the projects undertaken. Rapid

isolation of valuable natural products from a variety of plant materials was further highlighted through isolation of castanospermine and marrubiin.

The simplicity of the method for bioprospecting/chemotaxonomic profiling in combination with near exhaustive product isolation was also further supported through the survey of *T. lanceolata* from Wielangta Forest and the rapid identification of major components from *D. winteri*. The rapid extraction, combined with the simplicity of the subsequent flash column chromatography (as demonstrated for polygodial from *T. lanceolata* in Chapter 2) allowed this project to be conducted in a more efficient manner than if a different extraction methodology were undertaken.

Finally, the use of the method as an alternative to hydrodistillation for the rapid generation of extracts rich in essential oils further highlights the flexibility and application of the method.

Chapter 4: Polygodial as a Scaffold for Synthesis

4.1 Overview

In this chapter, the reactivity of polygodial and derivatives was explored to investigate the potential utility of this molecule as a complex scaffold for organic synthesis. The fundamental reactivity of this compound was explored through various classical synthetic transformations. Subsequently, the development of a reactant for the divergent synthesis of known natural products and analogues based on polygodial was explored.

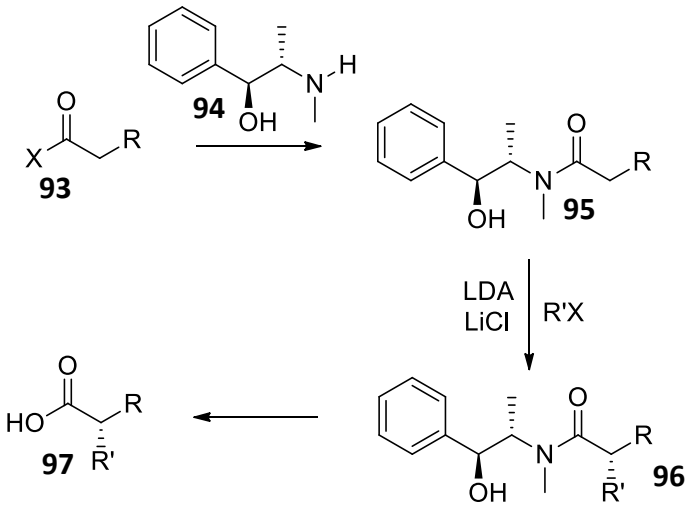
4.2 Background

4.2.1 Stereoselective and Stereospecific Synthesis

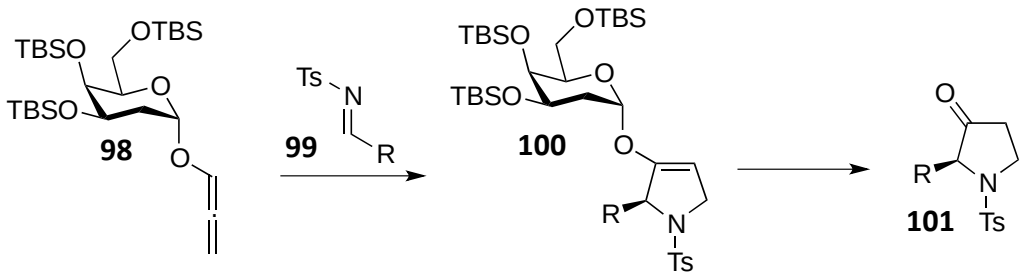
Stereoselective synthesis is of incredible importance in synthetic chemistry.[263] There are many examples of biologically active compounds that have only one active stereoisomer, which can mean a single enantiomer out of many possible stereoisomers.[264] For paclitaxel (**7**), described in Chapter 1, the 11 stereocentres result in a total of 2^{11} = 2048 possible stereoisomers. Stereoselective synthesis also leads to efficiency, as undesired by-products of differing stereochemistry are minimised or eliminated altogether.[265] Stereoselective synthesis can be achieved in a number of ways, but may suffer some drawbacks such as increased number of steps, low yield, low stereoselectivity, and/or expensive and sensitive catalysts and ligands.[266]

The use of natural products in synthesis can mitigate some of the problems of stereoselective synthesis. Many natural products contain a significant number of stereocentres within the molecule, and often exist as a single enantiomer from the natural source. This is certainly true of the examples of diosgenin (**3**) and paclitaxel (**7**) discussed in Chapter 1. Stereocontrolled synthesis in biological systems is typically achieved through enzymatic control of the biochemical pathways. Indeed, such is the power of enzymes, that they are being increasingly exploited for chemical synthesis, both by using microorganisms to effect chemical transformations,[267] and by purifying enzymes and using these instead of more traditional catalysts and reagents to facilitate synthetic transformations.[268]

If a stereochemically pure complex scaffold is used as a starting point in a synthesis, this can avoid some of the problems associated with stereoselective synthesis. Complex scaffolds with fixed absolute stereochemistry can lead to stereospecific synthesis by imparting stereocontrol on subsequent transformations, as the steric bulk and set conformation of the molecule can force reactions to occur only in a specific orientation leading to a single stereoisomer being formed. The complex scaffold may be an intrinsic part of the desired molecule, or alternatively a bulky chiral fragment may be attached to an achiral molecule, and subsequently removed from the substrate after the reaction. In the case where the chiral portion is removed, it is known as a chiral auxiliary. Of course, this specificity can also be a disadvantage as the enantiomeric series may not be able to be accessed when only a single enantiomer of a natural product is available. Two examples of natural products and derivatives used as chiral auxiliaries are pseudoephedrine (**94**) in the stereospecific synthesis of various carbonyl derivatives (**97**)[269] and carbohydrate derivatives (**98**) in the stereospecific synthesis of pyrrolidines (**101**).[270]



Scheme 4.1 – Asymmetric synthesis of carbonyl derivatives employing pseudoephedrine as a chiral auxiliary.



Scheme 4.2 – Asymmetric synthesis of pyrrolidines exploiting a carbohydrate derivative as a chiral auxiliary.

4.2.2 Drimane-Based Natural Products

This chapter is focused around the reactivity of polygodial, and the use of polygodial as a reactant for divergent synthesis of molecules containing the drimane skeleton. Drimane is a C_{15} terpene (sesquiterpene) found ubiquitously in nature as the core of many biologically active secondary metabolites.[271] Drimane (**102**) has the fixed absolute stereochemistry and numbering system shown in Figure 4.1.

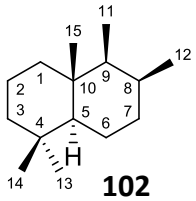
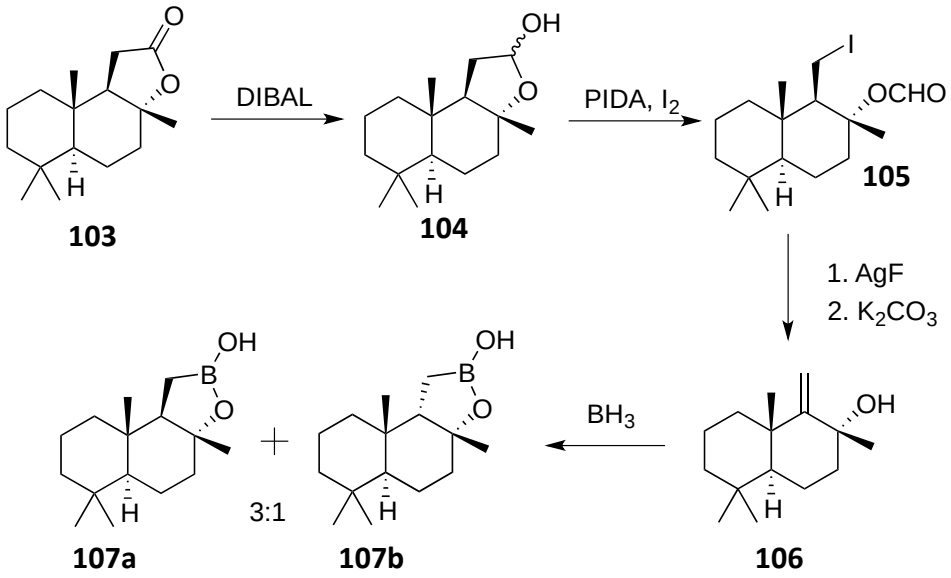


Figure 4.1 – Drimane skeleton with numbering system.

In 2012, work reported by Baran and co-workers highlighted the use of a similar complex organic scaffold, based on the drimane skeleton, which was used as a divergent precursor for the stereospecific synthesis of a suite of meroterpenoid natural products. The absolute stereochemistry of the drimane system allowed stereospecific reactions due to the bulk and rigid conformation of the scaffold. An inexpensive and readily available natural product, sclareolide (**103**), was used as a complex organic scaffold, and through a 5–step process generated borono-sclareolide (**107a**) as a key intermediate, that facilitated the divergent synthesis of numerous meroterpenoids.[272] This synthesis over 5 steps gave a 60 % yield of borono-sclareolide (**107a**), in a 3:1 ratio of diastereomers. From this intermediate, natural products such as (–)-isozonarol (**108**), (–)-isozonarone (**109**) and (–)-pelorol (**110**) were able to be efficiently synthesized.



Scheme 4.3 – Baran and co-workers' 5-step synthesis of borono-sclareolide

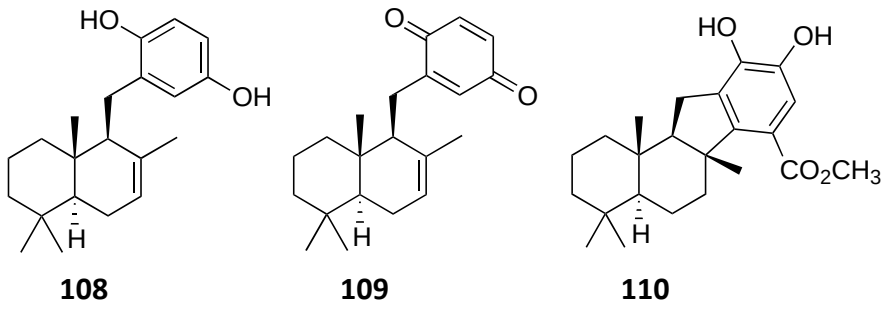


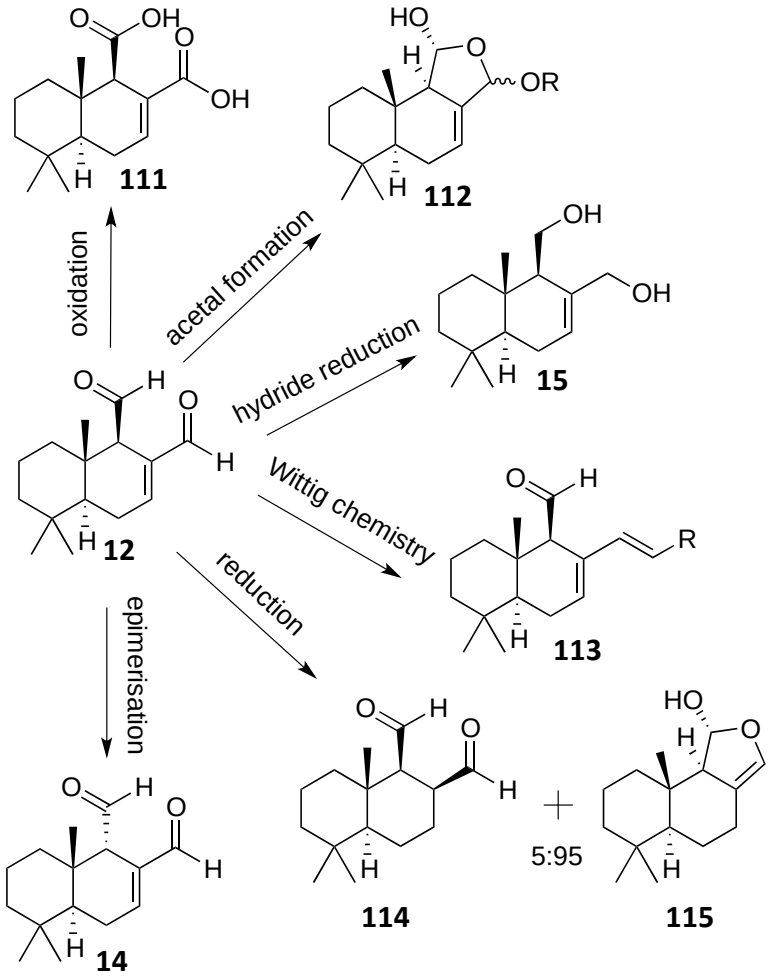
Figure 4.2 – (–)-isozonarol, (–)-isozonarone and (–)-pelorol synthesized from sclareolide.

Inspired by the work of Baran, efforts were made towards the synthesis of natural and non-natural compounds, starting from polygodial. The reactivity of polygodial and derivatives was explored through classical chemical transformations. Subsequently, the ultimate goal was to convert this valuable material into a substrate from which divergent synthesis of terpenoid natural products could be achieved.

4.3 Reactivity Studies on Polygodial and Derivatives

Currently, there is limited literature on the synthetic transformations of polygodial (**12**). Previously, there have been a number of reports of hydride reduction to form drimendiol (**15**),[76,241,273,274] and a report of the oxidation to the di-carboxylic acid (**111**).[275] In

addition, Kornienko and co-workers have reported a number of reactions of this scaffold including acetal formation (**112**) through reaction with various alcohols, hydrogenation with a nickel catalyst (**114** and **115**), epimerisation of the C-9 position to form 9-epipolygodial (**14**) and selective Wittig derivatisation of the conjugated carbonyl (**113**). Kornienko's work was reported while the current project was being undertaken.[276,277]



Scheme 4.4 – Previously reported transformations on polygodial.

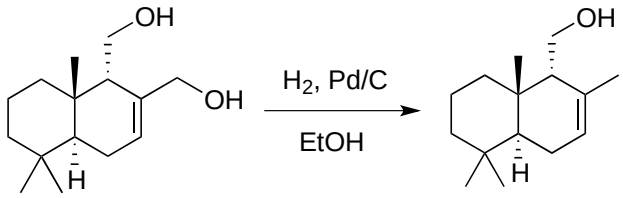
Polygodial (**12**) contains 3 contiguous stereocentres, and 2 distinct aldehydes. The carbon at C-9 adjacent the aldehyde at C-11 is epimerisable, and due to the steric and electronic difference, the aldehydes are able to be selectively functionalised. This makes polygodial an excellent candidate as a synthetic scaffold towards natural products and analogues. Polygodial is expensive to source commercially, which limits its feasibility as a precursor for synthetic transformations if an alternative source is not available.* However, as described in Chapter 2, gram-scale quantities of pure polygodial can be isolated without

* AUD$162 for 10 mg as at 29/10/17 from Sigma–Aldrich

chromatography within a day from *T. lanceolata* by the PHWE method, thus allowing polygodial to be a viable scaffold for synthesis.

4.3.1 Reduction of Drimendiol

Following the near quantitative conversion of polygodial (**12**) to drimendiol (**15**) (Chapter 2), further synthetic manipulations were performed to allow a greater understanding of the reactivity of this diol. A previous report on synthetic manipulation of the C-9 epimer of drimendiol (**116**) showed that when subjected to a hydrogen atmosphere with catalytic Pd/C, the resulting product was not the predicted hydrogenation product, but instead hydrogenolysis of the allylic alcohol occurred to form 9-epidrimenol (**117**).[273]



116 **117**

Scheme 4.5 – Hydrogenolysis of 9-epidrimendiol to form 9-epidrimenol

This reaction was explored on drimendiol, with the synthesis of (–)-drimenol (**118**) in mind. Upon reaction with hydrogen gas and Pd/C in EtOH for 1.25 h, (–)-drimenol was isolated in a reproducible 64 % yield. Key signals in the ^{1}H NMR spectrum for this compound were the diastereotopic methylene signals at 3.85 and 3.73 ppm, which indicated that the compound contained only one -CH_2-OH group. Further, the signal at 5.54 ppm indicated that the alkene was still intact. The spectral data were consistent with the formation of (–)-drimenol,[278] showing that the reaction proceeded in the same fashion as for the C-9 epimer previously reported. As (–)-drimenol is formed as the only product of the reaction, this indicates that the hydrogenolysis of the allyl alcohol occurs exclusively before any subsequent hydrogenation of the alkene. A possible mechanism is that this process occurs through the formation of a π-allyl cation.

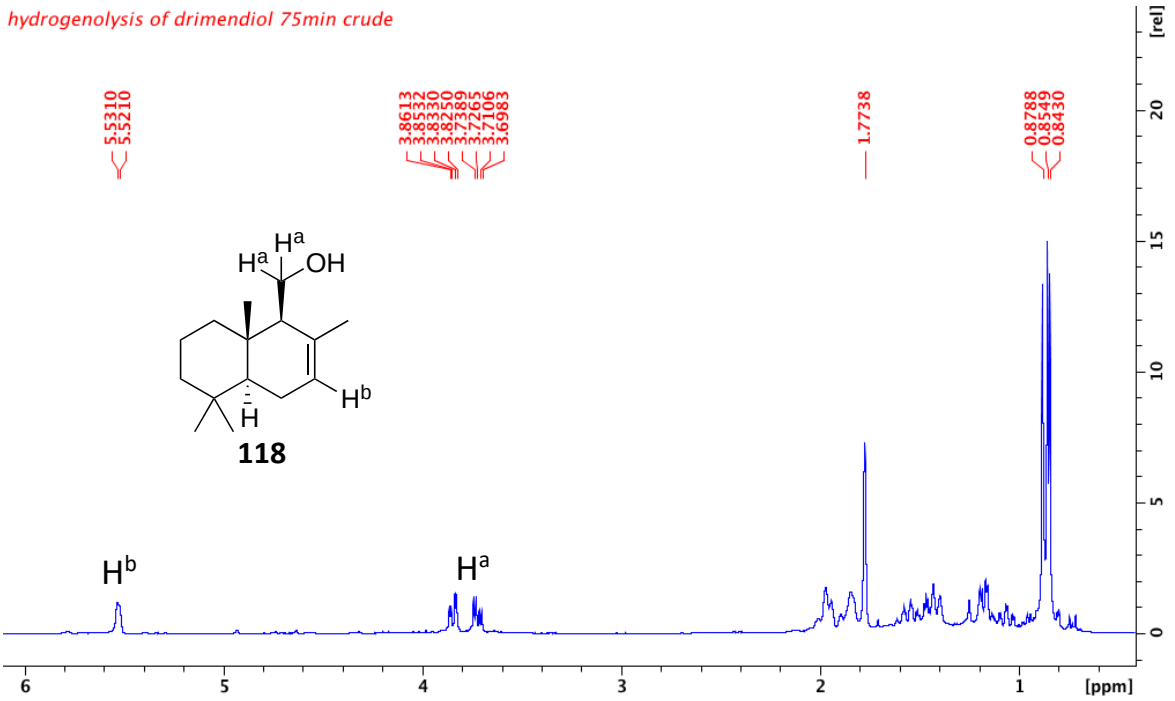
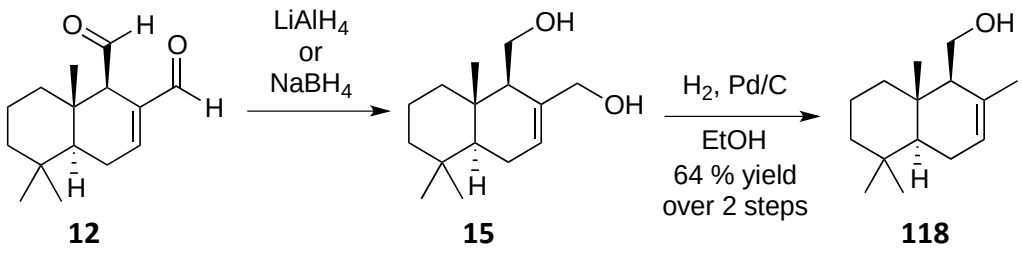


Figure 4.3 – ^{1}H NMR spectrum of (–)-drimenol (**118**).

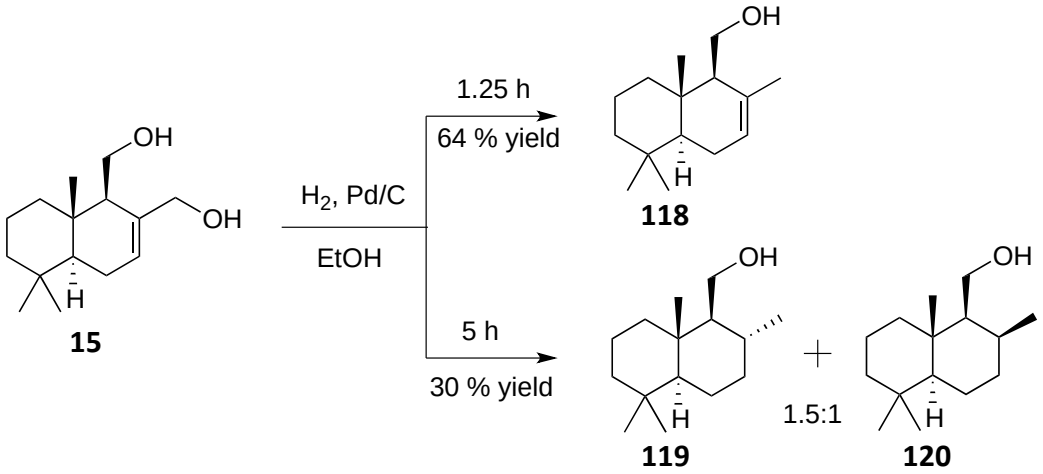


Scheme 4.6 – Two-step synthesis of (–)-drimenol from polygodial.

(–)-Drimenol (**118**) is a bench-stable compound, which has been used as a scaffold in synthesis of numerous natural products. To source (–)-drimenol commercially for synthesis is not viable due to it being prohibitively expensive,* so this method for obtaining the material provides a significant advantage. Gram-scale quantities of (–)-drimenol are obtained by this method in a reproducible yield of ~64 % over 2 steps, which represents the most efficient means of obtaining this compound to the author's knowledge. A review of the synthesis of (–)-drimenol, and its use as a scaffold for synthesis of natural products and derivatives was published in 2011.[279]

* USD$730 for 5 mg from Ark Pharm, USA 14/11/17

When the reaction time was increased to 5 h, 2 major products were observed, neither of which were (–)-drimenol (**118**). In the ^{1}H NMR spectra for these two products (separable by flash chromatography), the appearance of methyl signals split into doublets indicated that the hydrogenation of the double bond was occurring, forming the two epimeric saturated drimanols **119** and **120**, whose NMR spectroscopic data matched literature reports.[280,281] The low yield (~30 % combined) was consistently observed with this particular reaction, indicating that the compound degrades over time under these conditions, forming unknown by-products that were not isolated or identified. This reaction showed a 1.5:1 ratio of **119** to **120**, suggesting some stereocontrol for this reaction imparted by the configuration of the drimane backbone.

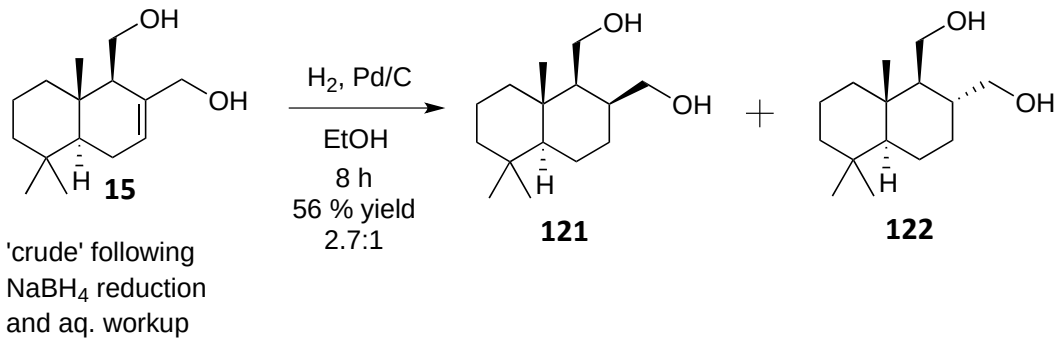


Scheme 4.7 – Hydrogenolysis and hydrogenation of drimendiol **15**.

Surprisingly, reduction of crude drimendiol following reduction of polygodial with $NaBH_4$ resulted in the isolation of the two isomers of drimandiol **121** and **122**. The only difference to previous reactions was that the drimendiol **15** was the crude reaction product, and not purified by column chromatography. This reproducible observation indicated that the crude $NaBH_4$ reaction product contained a contaminant that was hindering the hydrogenolysis step.

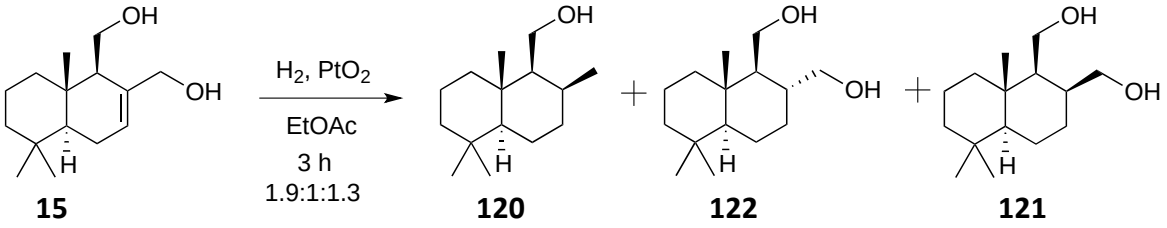
As boron is oxophillic it was hypothesised that residual boric acid may be preventing formation of a π-allyl cation and therefore preventing hydrogenolysis. To test this, the same reduction conditions were attempted on the pure diol with the addition of trimethylborate. However, this reaction proceeded to form the two saturated drimanol isomers **119** and **120**. Therefore, it is not clear why there is a stark contrast between

using the crude and pure drimendiol. This was not explored further, but is potentially useful to target the dihydroxy derivatives **121** and **122**.



Scheme 4.8 – Hydrogenation of crude drimendiol.

Drimendiol was also reacted with Adam's catalyst (platinum oxide, PtO_2) under 1 atm of hydrogen in EtOAc for 3 h. Following flash chromatography, 3 compounds were isolated - *syn*-drimanol **120** and drimandiol isomers **121** and **122**. This reaction shows that under these reduction conditions, there is competition between hydrogenation and hydrogenolysis, which resulted in an almost 1:1 mixture of the drimanol isomer **120**, with the drimandiol isomers **121** and **122**. *syn*-Isomer **121** has been previously reported in the literature,[282] however, *anti*-isomer **122** has not previously been fully characterised. The key features in the NMR spectra for diol isomers **121** and **122** are the loss of any alkene signal, and the two sets of diastereotopic methylene signals observed between 3.5 and 4 ppm.



Scheme 4.9 - Reduction of drimendiol with H_2/PtO_2 in EtOAc

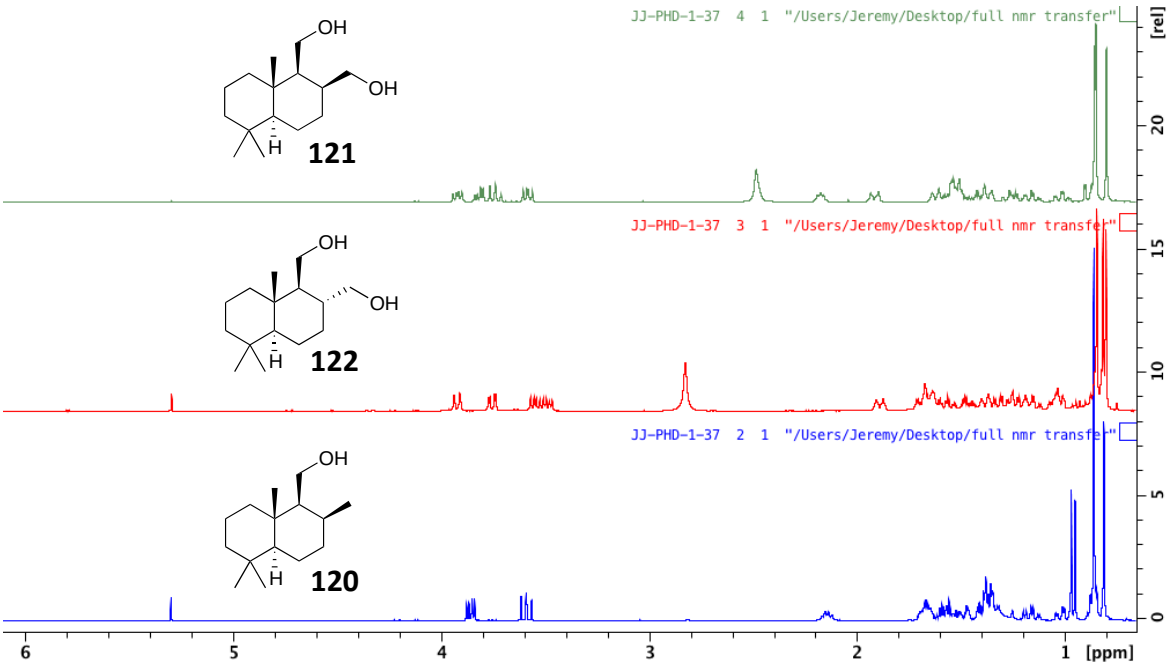


Figure 4.4 – ^{1}H NMR spectrum of the products from reduction of drimendiol with H_2/PtO_2 in EtOAc

To support the stereochemical assignment, diols **121** and **122** were crystallised, and analysed by single crystal X-ray diffraction. The difference in the stereochemistry at the C-8 position was supported through this analysis and matches with the stereochemistry previously reported for the *syn*-isomer **121**.[282] The bias towards isomer **121** is supported by the X-ray crystal structure 3D conformation of the starting material drimendiol, as the -CH_2OH at C-9, and the methyl group at C-10 provide steric bulk near C-8, which favours delivery of hydrogen by the catalyst on the opposite face.

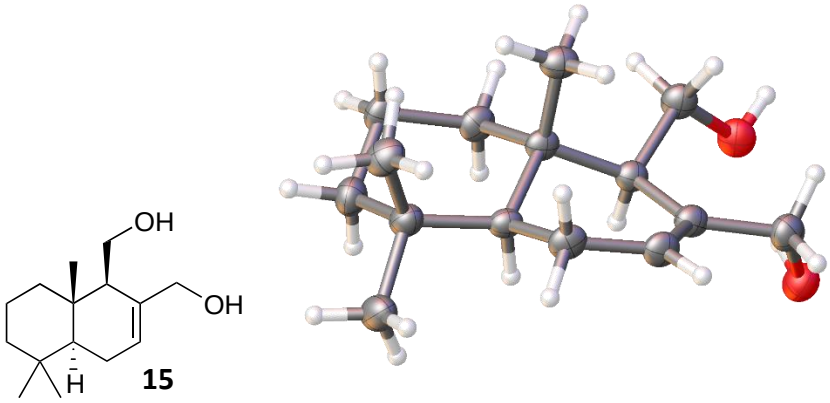


Figure 4.5 – X-ray crystal structure of drimendiol **15**.

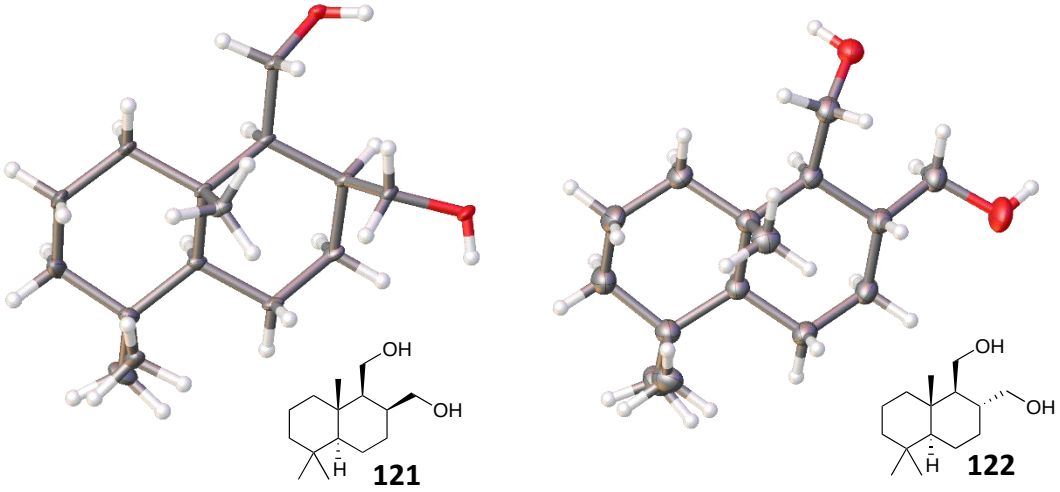


Figure 4.6 – X-ray crystal structures for the saturated diols **121** (left) and **122** (right).

4.3.2 Hydrogenation of Polygodial

In continuing work on the reactivity of polygodial (**12**), reduction under various conditions with hydrogen was attempted. Polygodial was subjected to a hydrogen atmosphere with catalytic Pd/C in both EtOH and EtOAc. ^{1}H NMR spectroscopic analysis of the crude material obtained from the reactions indicated a complicated mixture of products in each case. The ^{1}H NMR spectrum of the reaction in EtOAc shows a significant number of aldehyde signals, showing at least 3 major products in this reaction. Additionally, the signals around 4 ppm are consistent with formation of various acetal type derivatives. This is consistent with the result seen by Kornienko and co-workers using a nickel catalyst.[276] As a major compound was not observed, no further reactions were attempted.

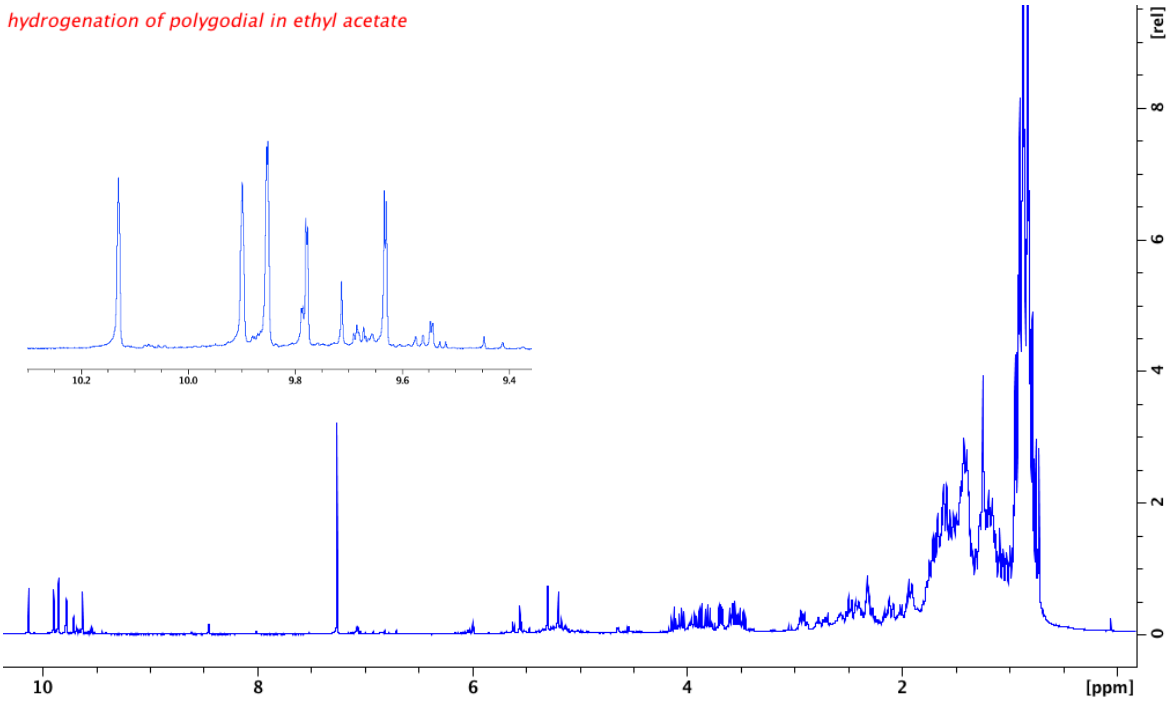
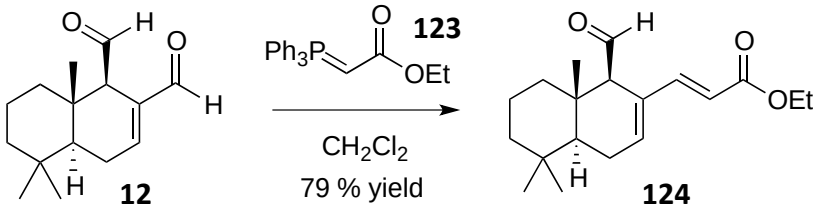


Figure 4.7 – ^{1}H NMR spectrum of hydrogenation of polygodial in EtOAc.

4.3.3 Olefination of Polygodial

Reaction of polygodial (**12**) with the Wittig reagent, ethyl (triphenylphosphoranylidene)acetate (**123**), resulted in regioselective reaction of the α,β-unsaturated aldehyde at C-12 to form compound **124** in good yield (79 %). Surprisingly, with an excess of the Wittig reagent, the aldehyde at C-11 aldehyde showed no tendency to react. This was postulated to be due to the steric hindrance of the neo-pentyl aldehyde. This result was independently observed by Kornienko and co-workers, detailed in a report published around the same time as this work.[76] The ^{1}H NMR spectrum of the purified material clearly shows the remaining aldehyde with a doublet at 9.46 ppm, supporting the selectivity of the reaction for the C-12 aldehyde of polygodial. The expected formation of the (*E*)–alkene was supported in the ^{1}H NMR spectrum by the 16.2 Hz coupling constant between the signals of the newly formed alkene protons at 7.30 and 5.48 ppm.



Scheme 4.10 – Wittig reaction of polygodial (**12**)

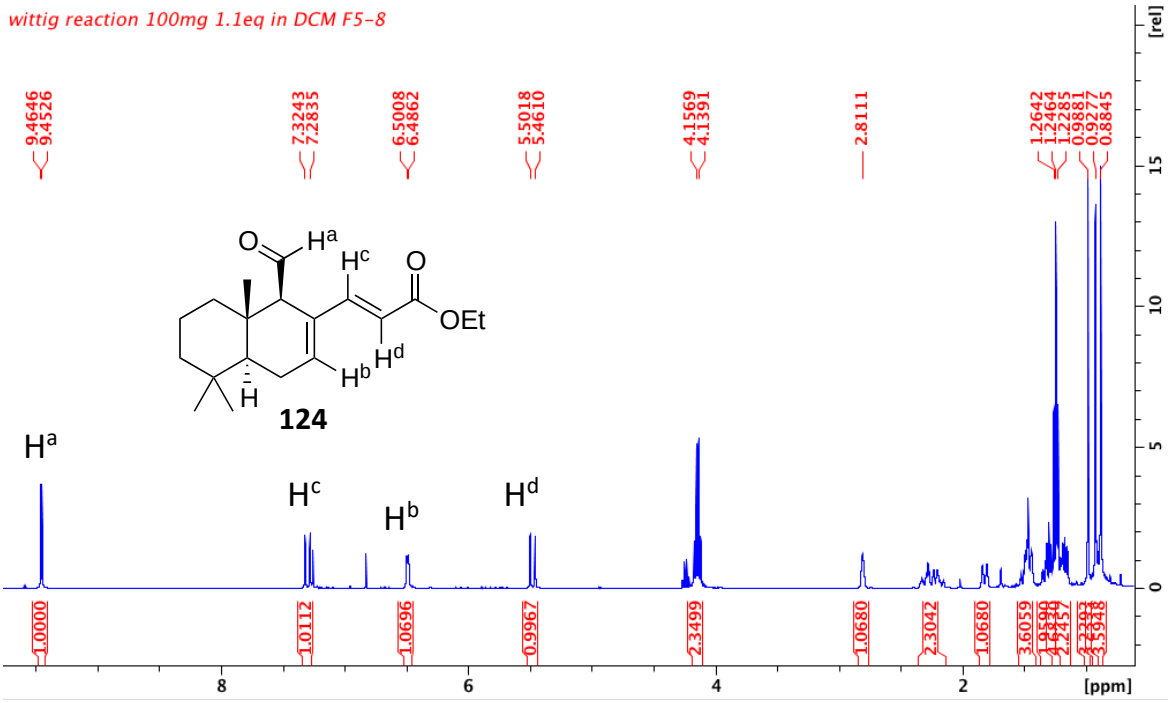
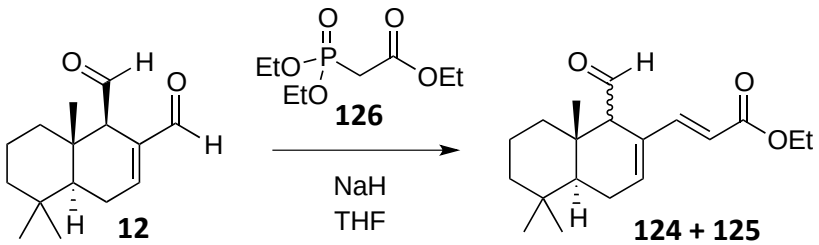


Figure 4.8 – ^{1}H NMR spectrum of the purified Wittig product **124**.

A Horner–Wadsworth–Emmons olefination was attempted, where polygodial was reacted with triethyl phosphonoacetate (**126**) and sodium hydride in THF. This reaction resulted in a mixture of products with analysis of the ^{1}H NMR spectral data indicating an inseparable mixture of the expected acrylate product with the C-9 epimer **125** indicated by a second doublet at 9.53 ppm in the ^{1}H NMR spectrum, as well as further by-products.



Scheme 4.11 – Horner–Wadsworth–Emmons Reaction of polygodial.

4.3.4 Reactivity of the Wittig Adduct

This Wittig product **124** compound contains a diene moiety, which may undergo Diels–Alder type chemistry. Therefore, this reactivity was explored to demonstrate the facile synthesis of enantiopure polycyclic systems. To this end, a model reaction using *N*-phenylmaleimide (**127**) was undertaken in an attempt to form a tricyclic carbon skeleton. This reaction proceeded to yield a single enantiomerically pure compound (**128**) in moderate yield (55 %). In spite of the moderate yield, no evidence for other stereoisomers was observed in the ^{1}H NMR spectral data. The structure was supported through spectroscopic analysis, including HRMS consistent with the predicted structure. In the ^{1}H NMR spectrum, the resonance at 9.58 ppm showed that the aldehyde remained intact in the reaction, and the incorporation of a phenyl moiety was indicated by signals integrating for a total of 5 protons between 7 and 7.5 ppm. The presence of the nitrogen containing heterocycle was supported through the carbonyl signals at 176.6 and 175.7 in the ^{13}C NMR spectrum. The stereochemical assignment was supported through the NOE correlations shown in Figure 4.9, below, and is consistent with the expected outcome of a Diels–Alder reaction through an *endo*-transition state.

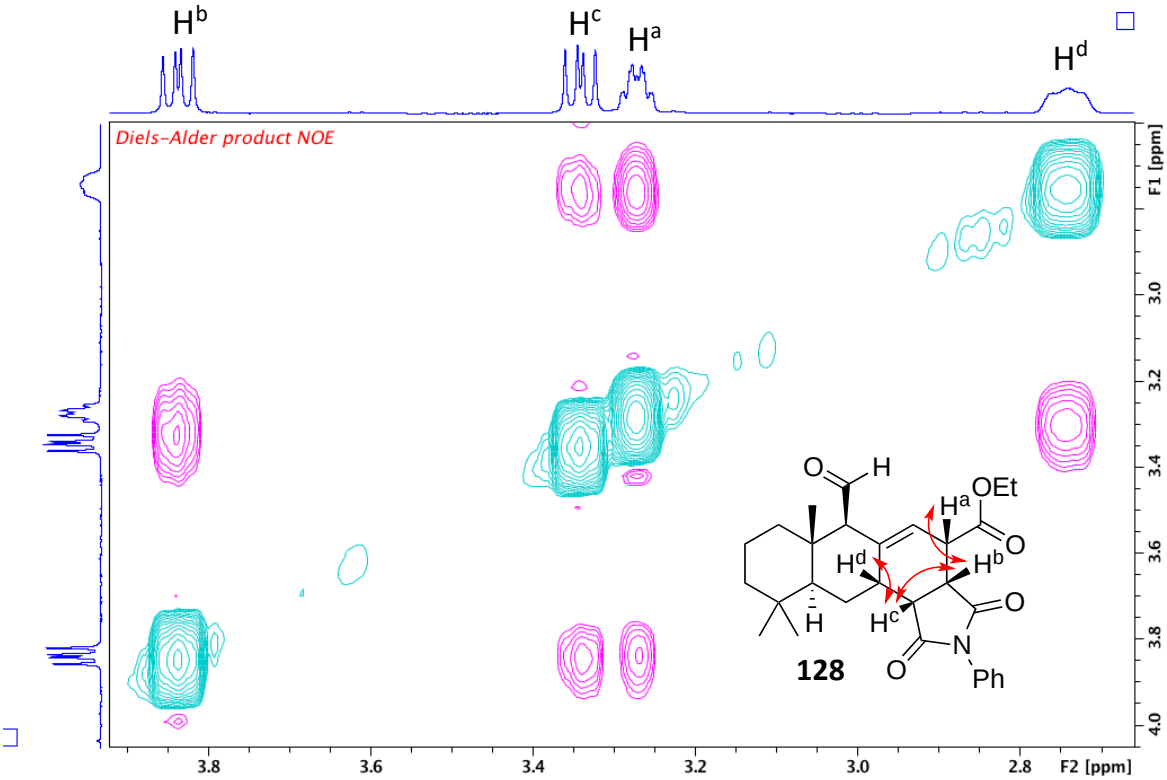
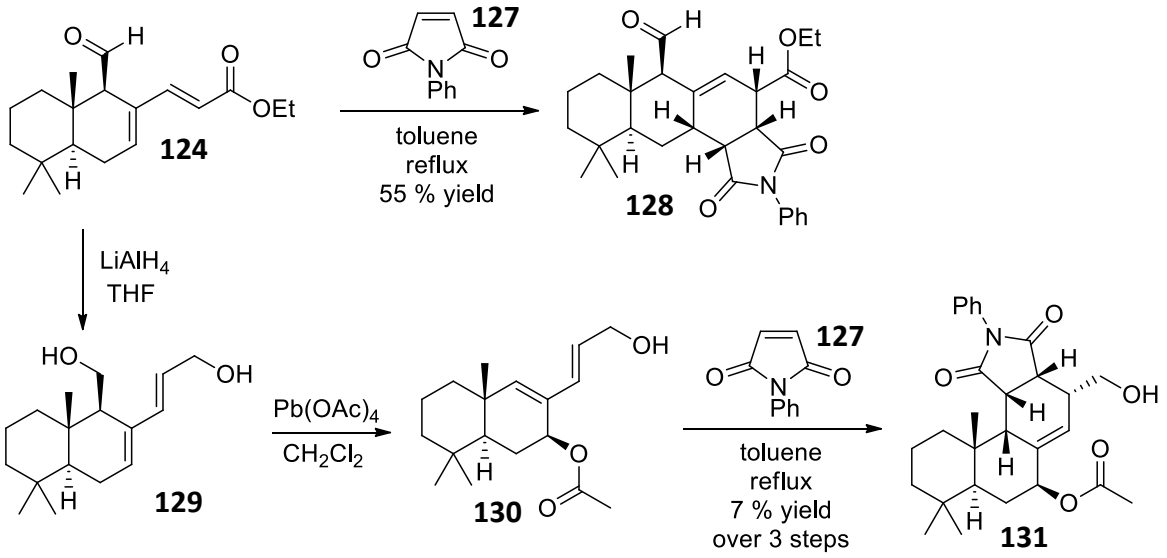


Figure 4.9 – Partial NOE Spectrum of compound **128**.

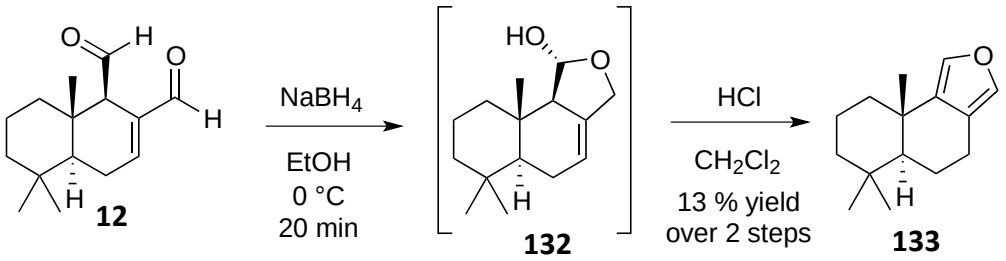
The Wittig adduct **124** was also subjected to reaction with $LiAlH_4$ to reduce the aldehyde and the ester functionalities, setting up for subsequent reaction to form a different diene substrate for synthesis of novel tricyclic compound **131**. This reaction proceeded to give a complicated mixture of products, from which an impure fraction containing the diol **129** was obtained in low yield. As the desired product was obtained in sufficient yield for further reaction, no optimisation was undertaken to improve the yield or purity of compound **129**. This fraction was then subjected to $Pb(OAc)_4$, which proceeded as expected based on analogous reactivity observed in similar systems by Preite and Cuellar[283] to form **130** in moderate yield, which contained a rearranged diene system which could undergo Diels–Alder chemistry. Diels–Alder reaction with **127** proceeded to yield polycyclic product **131** as a single diastereomer in 7 % yield over 3 steps. This reaction proceeded as expected with HRMS consistent with the expected product from a Diels–Alder reaction, and the NMR spectra again supporting the inclusion of the maleimide as discussed for compound **128**. In addition, a carbonyl signal at 170.6 ppm in the ^{13}C NMR spectrum, and a methyl singlet at 2.15 ppm (overlapped signal) in the ^{1}H NMR spectrum indicated the acetoxy functionality. This reaction leaves another synthetic handle in the form of an allylic acetoxy group at the C-7 position. This could be exploited in cross-coupling reactions with Pd,[284] or hydrolysed to yield an allylic alcohol which may also undergo a number of reactions including Pd-mediated dehydrative cross-coupling with alkenes.[285]



Scheme 4.12 – Synthesis of enantiopure polycyclic systems **128** and **131**.

4.3.5 Synthesis of (+)-Euryfuran

The reduction of polygodial with $NaBH_4$ in EtOH was investigated in more detail to determine if the compound isodrimeninol **132** could be formed through selective reduction of the α,β-unsaturated aldehyde. This would allow the synthesis of (+)-euryfuran (**133**), a marine natural product found in nudibranchs and sponges.[286,287] It was thought that the cyclic intermediate formed in the reduction would efficiently eliminate water in the presence of acid, followed by rearrangement of the alkene to aromatise the system, forming the furan. Initially, polygodial was reacted with 1.1eq of $NaBH_4$ at rt for 4 h. ^{1}H NMR spectroscopic analysis indicated the exclusive formation of drimendiol in this reaction. Performing the reaction at 0°C for 20 min gave the desired isodrimeninol (**132**) in 78% yield (containing some residual impurity). With this in hand it was treated with HCl to provide (+)-euryfuran (**133**) in a 13 % yield over 2 steps. The formation of isodrimeninol by oxidation of drimendiol (**15**) with Dess–Martin periodinane was also attempted as an alternative pathway to this natural product, however these reactions produced a complex mixture of products.



Scheme 4.13 – Synthesis of the natural product (+)-euryfuran via isodrimeninol.

The ^{1}H NMR spectrum of the intermediate, isodrimeninol (**132**), shows the C-7 – C-8 alkene of polygodial still in place with a signal at 5.54 ppm. The other key peaks for this intermediate are the diastereotopic methylene peaks for C-12 at 4.25 and 4.47 ppm. The C-11 acetal is also evident through the resonance at 5.25 ppm. Upon treatment with HCl, the resulting ^{1}H NMR spectrum for (+)-euryfuran showed the formation of the furan through the proton signals at 7.08 and 7.05 ppm, and loss of the C-7 – C-8 alkene signal. The appearance of diastereotopic methylene peaks at 2.77 and 2.50 ppm is consistent with the formation of the CH_2 at the C-7 position after the alkene shift. These data were consistent with literature reports.[288]

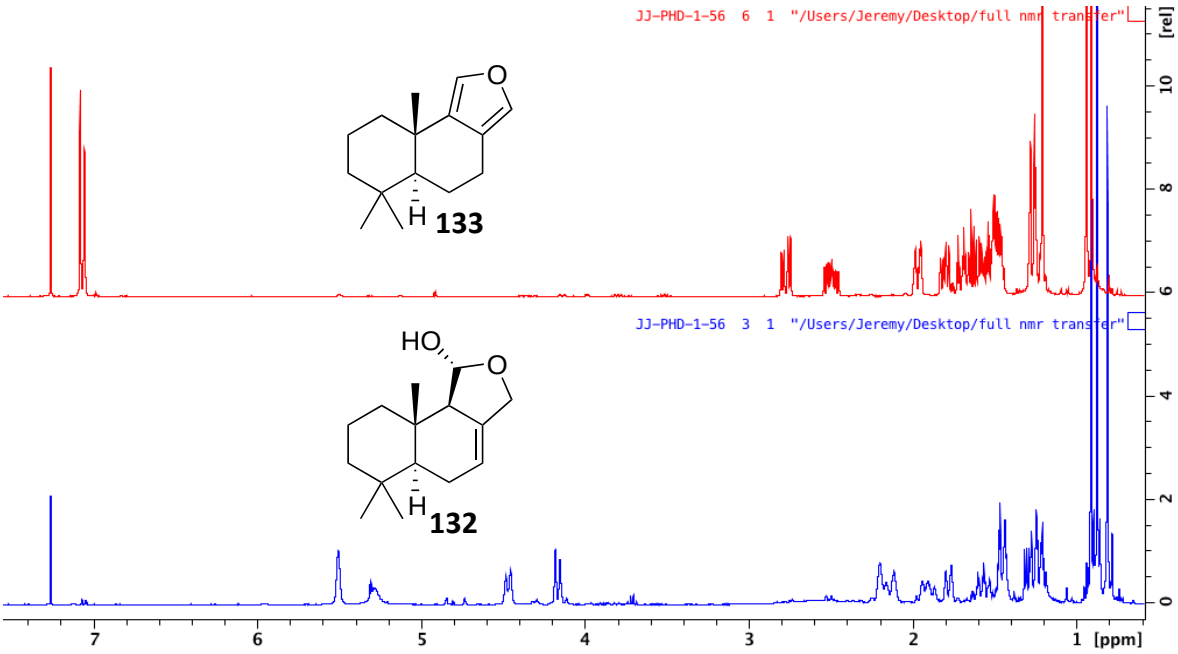
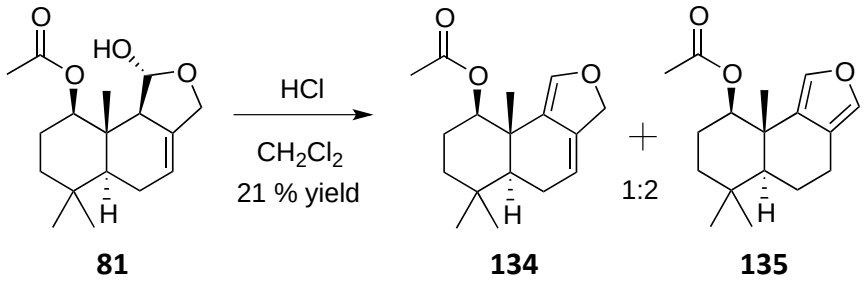


Figure 4.10 – ^{1}H NMR spectrum of impure isodrimeninol (**132)** (below) and (+)-euryfuran (**133**) (above).

4.3.6 Euryfuran Derivatives Synthesis

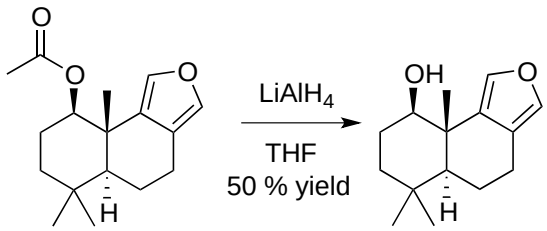
A hindrance in the aforementioned synthesis of (+)-euryfuran was the difficulty in obtaining the hemiacetal, isodrimeninol (**132**). In the previous report of the extraction and isolation of 1β-acetoxypolygodial (**80**) from a select group of *T. lanceolata* trees (Chapter 3), all of the specimens contained the corresponding 1β-acetoxyisodrimeninol (**81**). Treatment of **81** with HCl under analogous conditions to above yielded the corresponding furan analogue **135**. During this synthesis, intermediate **134** in which the alkene has not shifted into conjugation was also obtained and fully characterised. The ^{1}H NMR spectrum for **134** showed alkenyl peaks at 5.60 and 5.54 ppm, whereas the ^{1}H NMR spectrum for product **135** showed the formation of the furan through signals at 7.09 and 7.07 ppm and loss of the alkene signals. This reaction proceeded in low yield (21 %) with significant decomposition observed.



81 **134** **135**

Scheme 4.14 – Synthesis of 1β-acetoxyeuryfuran (**135**) and an intermediate (**134**) from the natural product 1β-acetoxyisodrimeninol (**81**).

Reaction of **135** with $LiAlH_4$ yielded the derivative 1β-hydroxyeuryfuran (**136**) in 50 % yield. This compound contains a hydroxyl group at C-1 as an extra synthetic handle to allow further modification of the compound.



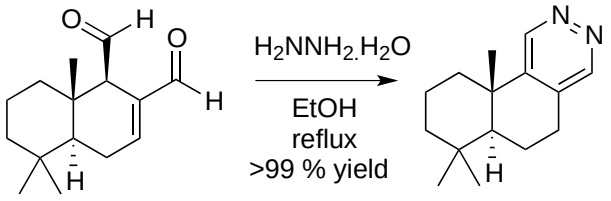
135 **136**

Scheme 4.15 – Reduction of 1β-acetoxyeuryfuran.

4.3.7 Pyridazine Synthesis

Continuing with studies on the reactivity of polygodial (**12**), a reaction with hydrazine hydrate was undertaken to observe whether the dialdehyde would undergo a double imine condensation to form a pyridazine. It was hypothesised for this reaction the alkene of the scaffold could shift into the newly formed ring to form the pyridazine system. As the C-11 aldehyde showed previously a lower reactivity than the C-12 aldehyde, it was not known whether this reaction would proceed on both aldehydes. However, as the second imine condensation would be intramolecular, this may increase the likelihood of the reaction occurring as anticipated. Polygodial (**12**) was reacted with 1.1eq of hydrazine hydrate in ethanol at rt for 3 h, resulting in a complex mixture of products, which indicated partial reaction.

Therefore, the reaction with excess hydrazine hydrate in refluxing ethanol for 1.5 h was attempted, which gave the desired pyridazine **137** in >99 % yield. The characteristic peaks in the ^{1}H NMR spectrum for the product are the singlets at 8.80 and 8.91 ppm corresponding to the two protons in the pyridazine ring. Recrystallisation of the compound provided crystals suitable for single crystal X-ray diffraction, which provided further supporting data for the assigned structure.



12 **137**

Scheme 4.16 – Pyridazine synthesis from polygodial.

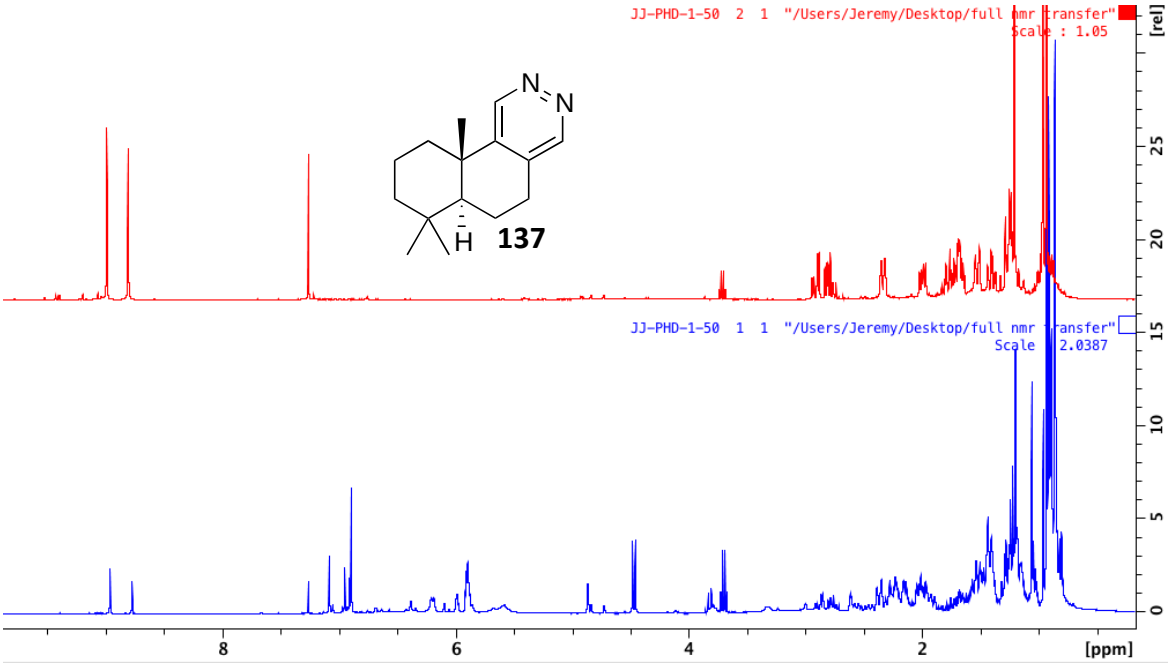


Figure 4.11 - ^{1}H NMR spectrum of the crude reaction mixtures of polygodial with hydrazine at rt (below) and reflux (above).

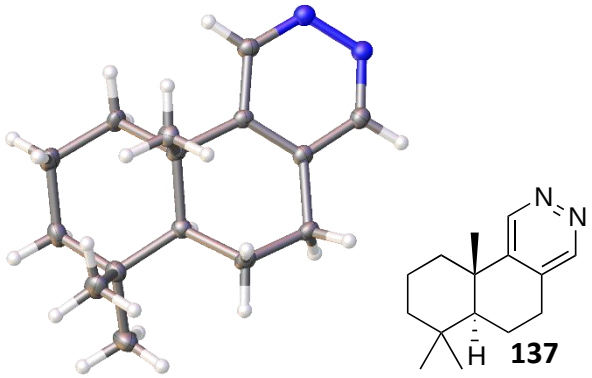
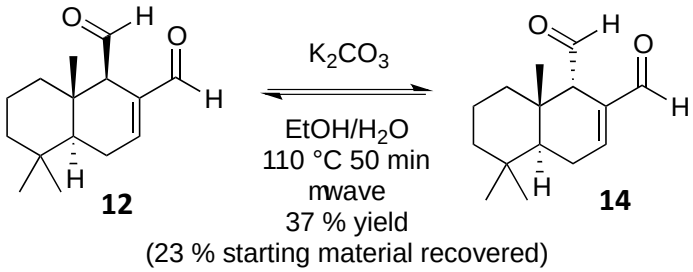


Figure 4.12 – X-ray crystal structure diagram of pyridazine **137**.

4.3.8 Epimerisation

9-Epipolygodial (**14**) has been reported to have interesting biological activity, however, the previously reported transformation was inefficient, with acid-catalysed epimerisation in toluene over 40 h providing the compound in 40 % yield.[276] Therefore, efforts were made to improve on the conditions and yield of the reaction. Given the previously observed epimerisation of polygodial at high temperature during PHWE extraction,[76] an attempt was made to effect the epimerisation through high temperature microwave conditions in a solution of 20 % EtOH:H_2O. The reaction was maintained at 110 °C for 20 min, but analysis of the ^{1}H NMR spectrum showed that the reaction had not proceeded, and the starting material (**12**) was recovered. Subsequently, addition of K_2CO_3 to the reaction showed that epimerization will proceed under microwave conditions in aqueous EtOH with the addition of base. Ultimately, the reaction proceeded in a reproducible 37 % yield after 50 min, with the recovery of 23 % starting material.

It was determined that the reaction reaches an equilibrium, which slightly favours the formation of 9-epipolygodial (**14**) under these conditions. Reaction times longer than 50 min decreased the overall yield presumably through decomposition and did not improve the ratio of the products. This reaction does not provide a higher yield than the previously reported synthesis, but does significantly decrease the reaction time. Crystals of 9-epipolygodial were formed, which were analysed by single crystal X-ray diffraction, and show clearly the change in position of the C-9 aldehyde to the opposite face of the ring, now in a pseudo–axial position, in contrast with the pseudo–equatorial position seen in the X-ray crystal structure of polygodial.



Scheme 4.23 – Reversible epimerisation of polygodial

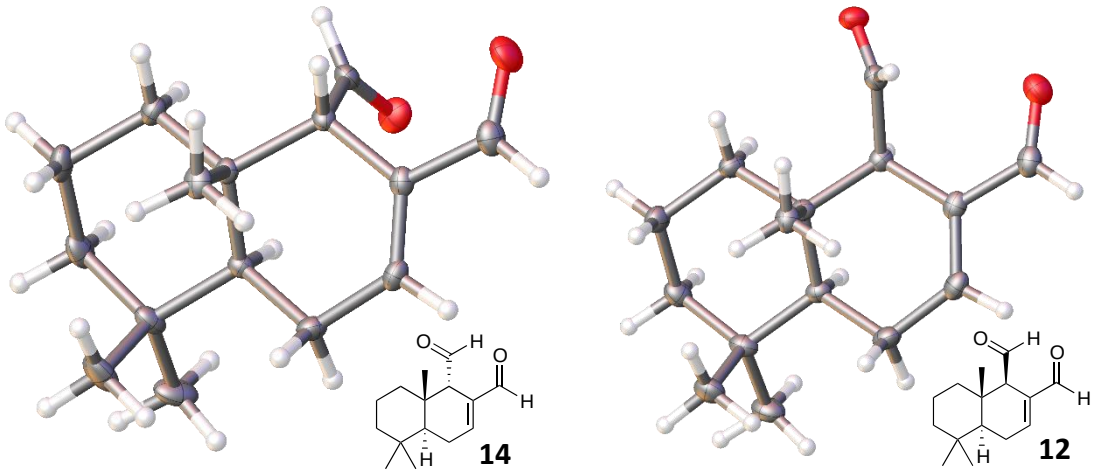
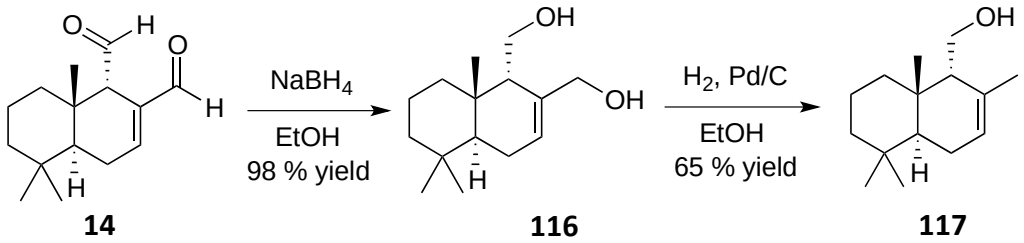


Figure 4.13 – X-ray crystal structure for 9-epipolygodial (left) vs polygodial (right)

The corresponding 9-epidrimendiol (**116**) and 9-epidrimenol (**117**) were synthesised from 9-epipolygodial (**14**) in yields almost identical to those seen for drimendiol (**15**) and (–)-drimenol (**118**), discussed previously. 9-Epidrimendiol (**116**) was crystallised and analysed by single crystal X-ray diffraction, providing further supporting evidence for the successful epimerisation and assignment of absolute stereochemistry.



Scheme 4.24 – Synthesis of 9-epidrimeniol (**116**) and 9-epidrimenol (**117**) from 9-epipolygodial (**14**).

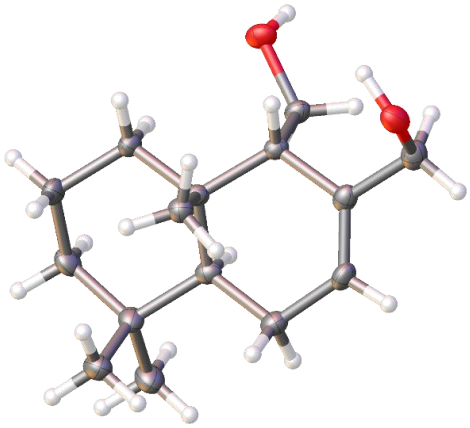
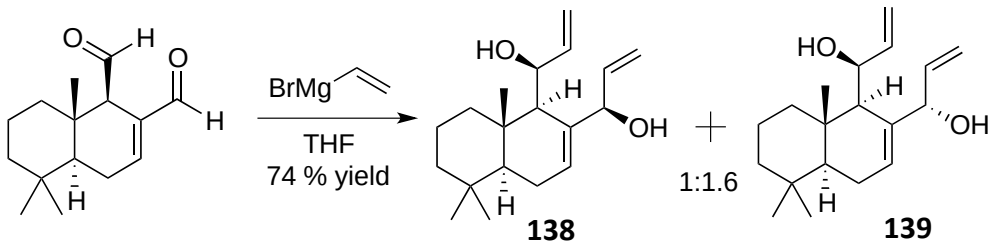


Figure 4.14 – X-ray crystal structure of 9-epidrimendiol **116**.

4.3.9 Polygodial Vinyl Grignard Reaction

Due to the observed lack of reactivity of the C-11 aldehyde of polygodial (**12**) towards the Wittig reagent **123**, a stronger nucleophile was employed to further investigate the different reactivity of the two aldehydes. Polygodial was reacted with excess vinylmagnesium bromide to yield two diastereomers **138** and **139**, which were separable by flash column chromatography. The saturated aldehyde at the C-11 position reacts with a high level of stereocontrol upon reaction with Grignard reagents (discussed in section 4.4.4) whereas the aldehyde at the C-12 position does not show the same level of control. Recrystallisation of diastereomer **139** provided crystals appropriate for single crystal X-ray diffraction, which provided supporting evidence for the assignment of stereochemistry. The ratio of the products was 1:1.6, shown by integration of the ^{1}H NMR resonances at 4.94 and 4.84 ppm, with an overall yield of 74 %.



Scheme 4.17 – Grignard reaction of polygodial with vinylmagnesium bromide

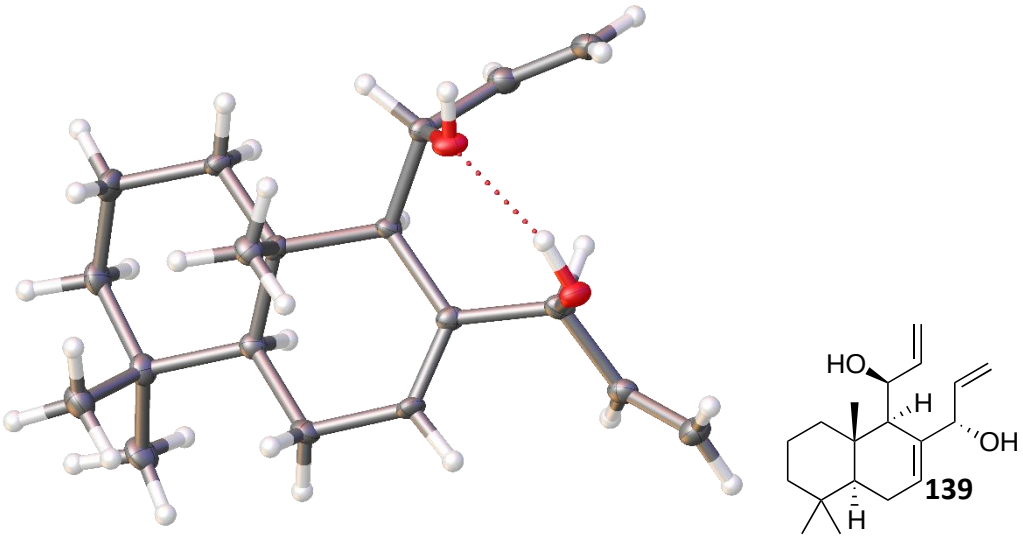


Figure 4.15 – X-ray crystal structure of the divinyl product **139**

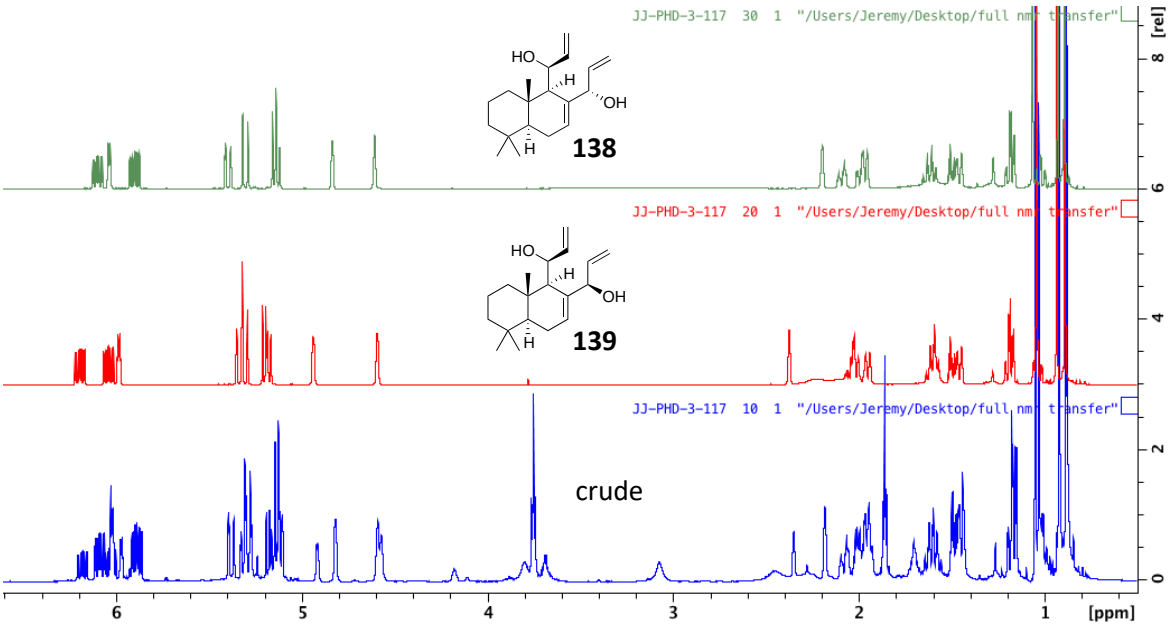
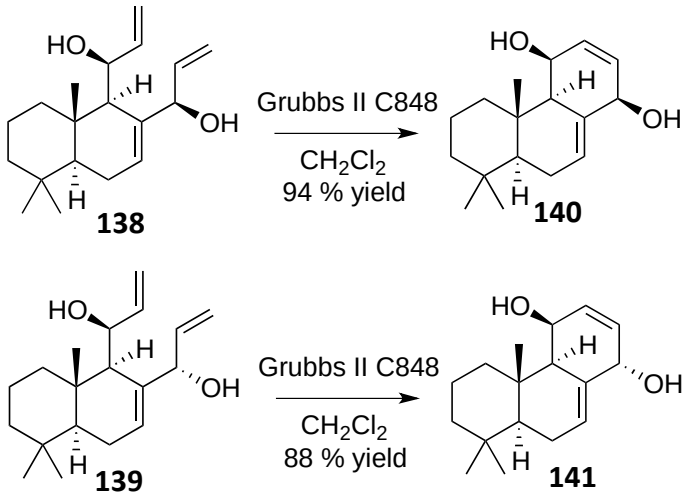


Figure 4.16 – ^{1}H NMR spectrum of the polygodial vinylMgBr reaction crude mixture (below) and the two isolated diastereomers **138** and **139** (above).

4.3.10 Ring-Closing Metathesis and Oxidation

Following the vinyl Grignard addition reaction, the resulting dienes **138** and **139** were subjected to ring-closing metathesis conditions to form tricyclic products **140** and **141**. Both of the stereoisomers proceeded efficiently in this reaction, with full conversion of

the starting material evident by TLC analysis after < 10 min with Grubbs 2nd generation catalyst C848 (10% catalyst loading), in CH_2Cl_2 at room temperature.



Scheme 4.18 – Ring-closing metathesis of divinyl drimane derivatives **138** and **139**.

In the ^{1}H spectra NMR for these compounds, the loss of the terminal alkene signals of the vinyl groups were indicative of a successful reaction. The ^{13}C NMR spectra also showed only 4 alkenyl signals for the products **140** and **141**, compared with 6 for the starting materials **138** and **139**.

Following successful RCM, oxidation with Dess–Martin periodinane was attempted to form a quinone, which ultimately resulted in the isolation and identification of quinone **143**. It was hypothesised that upon oxidation, the alkene from the drimane skeleton may shift into conjugation as was seen previously in the synthesis of (+)-euryfuran **133**, and pyridazine derivative **137**. An initial attempt at oxidation of tricyclic diols **140** and **141** with Dess-Martin periodinane gave a mixture of products, and subsequent flash column chromatography gave 3 impure fractions. The ^{1}H NMR spectroscopic analysis suggested that a quinone product had been formed, with the key piece of evidence being the formation of two very close doublets at 6.59 ppm and 6.55 ppm (10.0 Hz coupling). This was also supported by the appearance of 2 carbonyl signals at 189.5 ppm and 187.7 ppm in the ^{13}C NMR spectrum. Other chromatographic fractions appeared to have intermediates where the alkene had not shifted, which were complex mixtures of products. The HSQCme spectrum for the quinone fraction was analysed, and was found to only contain 4 methylene signals as opposed to the expected 5. There was also a

carbon resonance at 62.7 ppm in the ^{13}C NMR spectrum which was unexpected based on the proposed structure of the product, but is consistent with a >C**H**-OH in the ring. This led to the tentative identification of the quinone product as having an extra hydroxyl functionality (**142**).

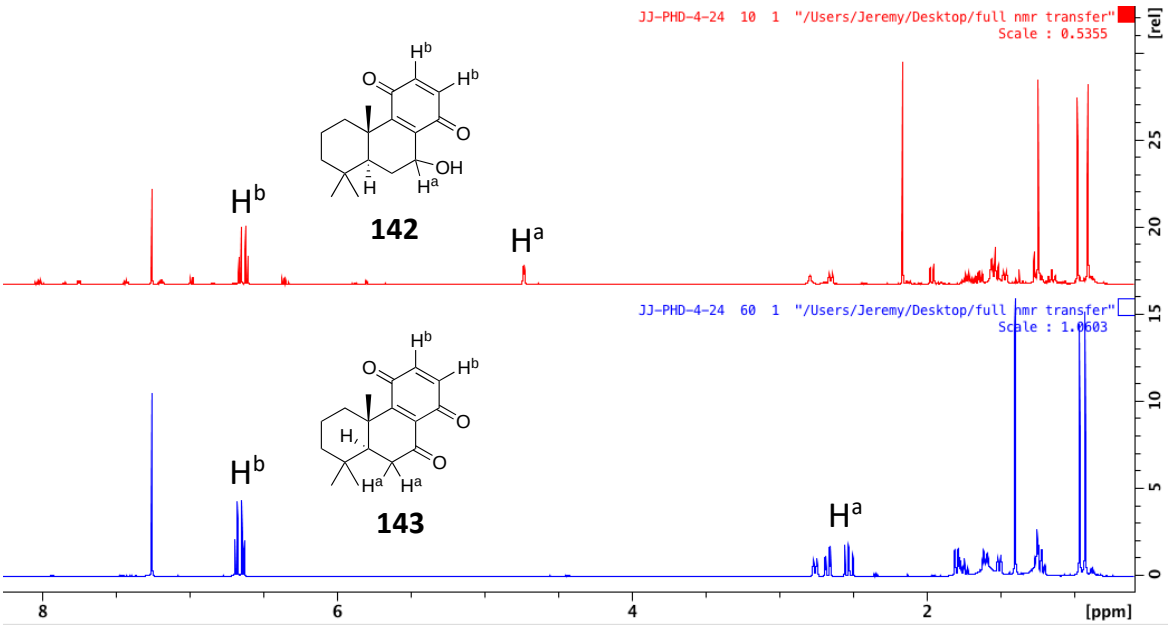
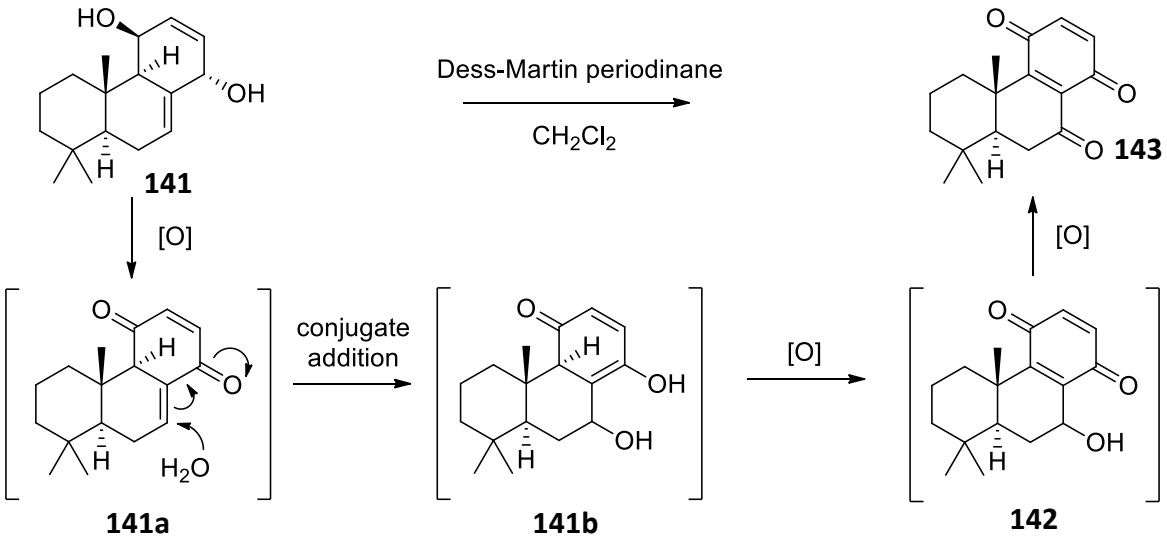


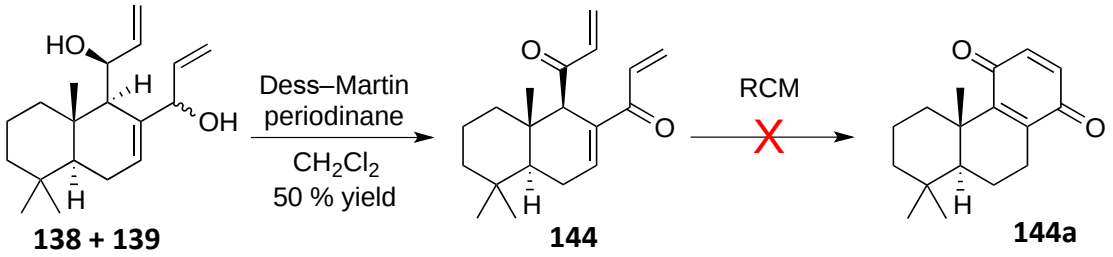
Figure 4.17 – ^{1}H NMR spectrum of the impure quinone fraction (above) and the purified compound after further oxidation (below)

This fraction was subjected to further reaction with Dess-Martin periodinane, and upon purification the ^{1}H NMR spectral data still showed evidence for a quinone, but the signals had shifted slightly to 6.71 ppm and 6.66 ppm. In addition, the ^{13}C NMR spectrum showed an extra carbonyl functionality at 197.0 ppm consistent with oxidation of the suggested alcohol to a ketone. This product was isolated and fully characterised as compound **143**. This shows that the reaction was able to form a quinone product, but suggests that under the given conditions this only proceeds upon nucleophilic attack of water onto the α,β-unsaturated carbonyl of the intermediate **141a** with subsequent tautomerisation and further oxidation to yield the α-keto quinone **143**. Understanding this process may allow optomisation to maximise the yield of the product, but this was not undertaken.



Scheme 4.19 – Quinone synthesis with an unexpected extra oxidation

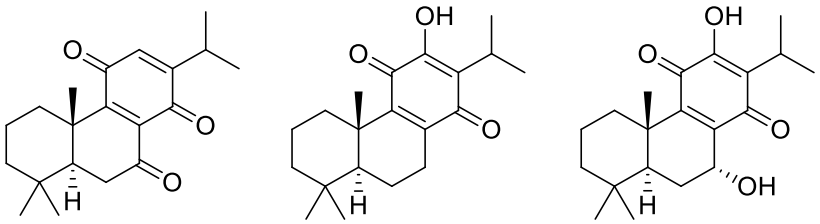
Oxidation of the system may also take place prior to the RCM reaction. To this end, oxidation of a mixture of the stereoisomers of the divinylpolygodial substrates **138** and **139** was undertaken and proceeded in 50 % yield to yield the diketone **144**. Subsequent attempts at RCM, however, in the manner described above provided complex mixtures.



Scheme 4.20 – Oxidation of divinylpolygodial **138** and **139** before ring-closing metathesis.

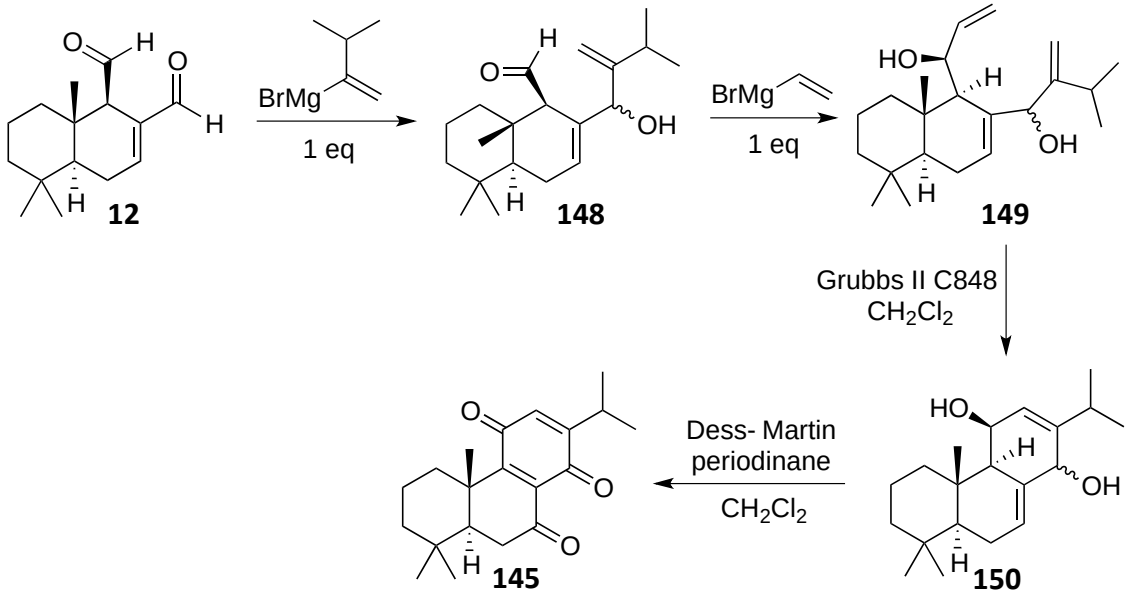
4.3.11 Proposed Future Work from RCM Reactions

As the reactivity of the two aldehydes of polygodial differ, as seen previously in Wittig type chemistry, this suggests that careful reaction control could allow for a first Grignard reaction to selectively functionalise the aldehyde at the C-8 position, followed by subsequent Grignard reaction to functionalise the aldehyde at the C-9 position. This, combined with the interesting result for the quinone synthesis above, could be used for the synthesis of the natural product cryptoquinone (**145**), an anti-fungal and cytotoxic diterpene,[289] as well as similar abietane diterpenes such as royleanone (**146**) and horminone (**147**) which have reported biological activity.[290-293]



145 **146** **147**

Scheme 4.21 – Abietane natural products cryptoquinone, royleanone and horminone

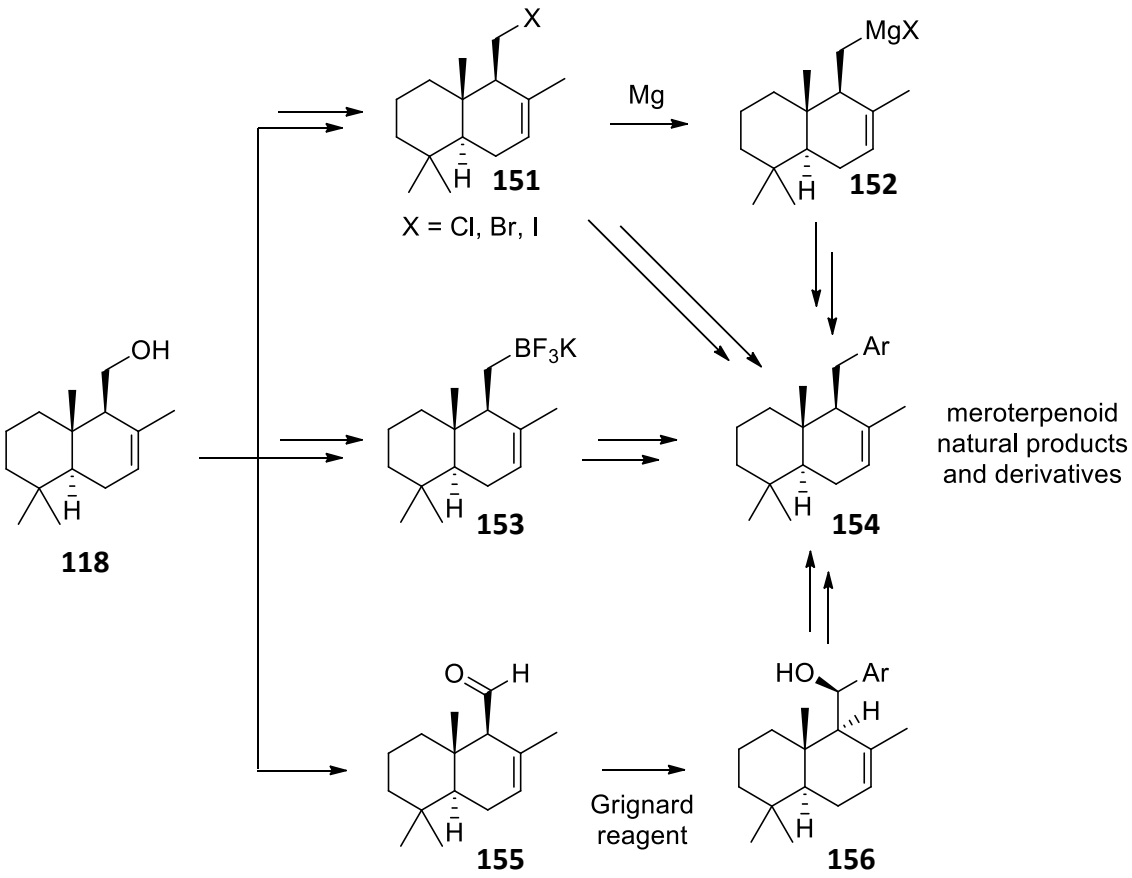


Scheme 4.22 – Proposed 4-step synthesis of cryptoquinone (**145**) from polygodial (**12**).

4.4 Towards a Reactant for Divergent Natural Product Synthesis from (–)-Drimenol

Inspired by Baran's work, development of a reactant based on polygodial for the divergent synthesis of natural products was investigated. The synthesis of various electrophilic and nucleophilic reactants was attempted. Initial work focussed on the halogenation of (–)-drimenol (**118**), and the saturated drimanol derivatives **119** and **120**. These can serve as electrophilic reactants in various coupling reactions, or be readily converted into a Grignard reagent through reaction with Mg metal to then subsequently react as a nucleophile with carbonyl or nitrile substrates. Following this, investigations towards development of a potassium alkyltrifluoroborate (R–BF_3K) salt (**153**) from polygodial (**12**) via (–)-drimenol (**118**) were undertaken. R–BF_3K salts are typically bench stable reagents which make excellent coupling partners for difficult alkyl transfers in Suzuki–Miyaura reactions.[294,295] Finally, investigations into electrophilic reactants in the

form of aldehydes and nitriles were conducted, which may react with Grignard or aryl lithium reagents. Ultimately, facile synthesis of the aldehyde (**155**) and subsequent Grignard reactions to form **156** proved the best route towards various natural product targets.



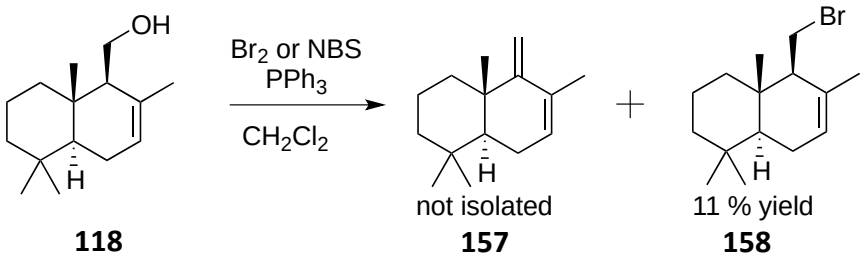
Scheme 4.25 – Proposed synthetic routes towards meroterpenoid natural products.

4.4.1 Functionalisation of (–)-drimenol

The reactivity of (–)-drimenol (**118**) towards forming these coupling partners was investigated. Whilst being a useful scaffold for synthesis as has been published previously,[279] (–)-drimenol provided some unanticipated challenges in attempts to functionalise it that have not been reported in the literature.

Halogenation of (–)-drimenol (**118**) for use as a coupling partner showed that the molecule is prone to elimination to form a conjugated diene derivative, drimendiene (**157**). In early tests, (–)-drimenol was halogenated with Br_2 and PPh_3, as well as *N*-bromosuccinimide and PPh_3. ^1H NMR spectroscopic analysis of the crude material showed a nearly identical composition of products. The 2 major products were diene **157**

formed through elimination, mentioned above, as well as the desired halogenation product **158**. In both cases these were formed in an ~1:1 ratio, with other minor products evident in the ^{1}H NMR spectra. Separation of these two products by column chromatography proved challenging due to their similar polarity, and only a portion of the compound was isolated to purity. The low yield, difficulty purifying, and sensitivity to elimination of the halide meant that transformation of this substrate was not pursued further.



Scheme 4.26 – Bromination and elimination of (–)-drimenol.

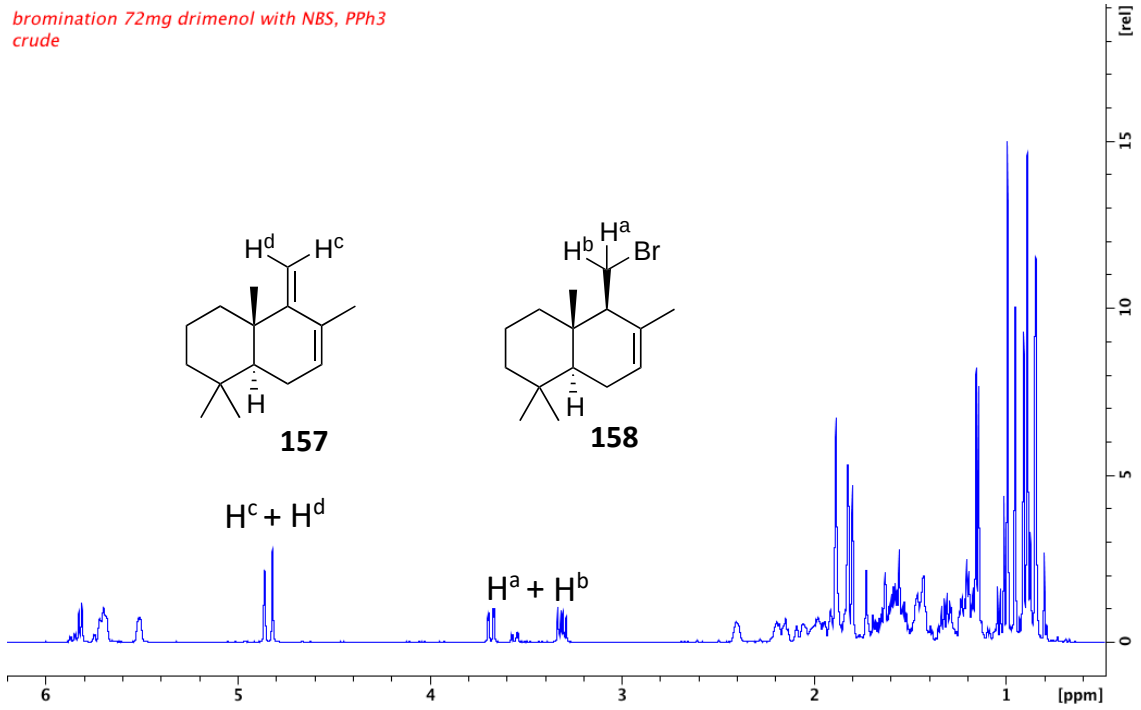
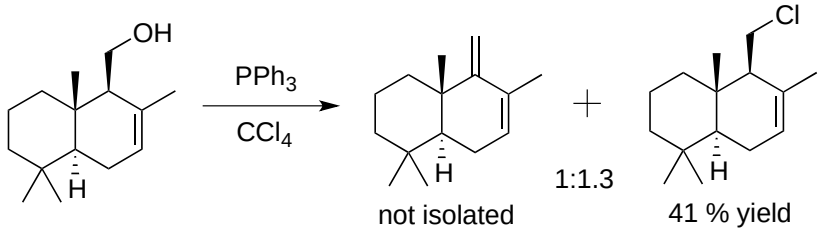


Figure 4.18 – ^{1}H NMR spectrum of the crude mixture from bromination of (–)-drimenol.

An Appel reaction was carried out, reacting (–)-drimenol with CCl_4 and PPh_3 to investigate the possibility of forming the corresponding chloride **159** without the elimination taking place. This reaction led to a similar result observed, with the ^{1}H NMR spectrum of the crude material showing a 1:1.3 ratio of the diene to the chloride. The

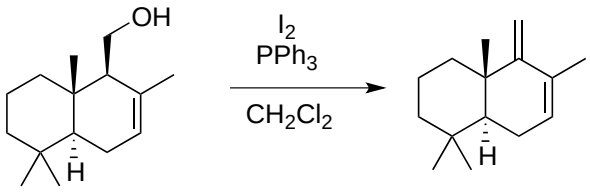
chloride was isolated from the reaction in 41 % yield, which represents a greater yield than the bromination, but was still an inefficient synthesis.



118 **157** **159**

Scheme 4.27 – Appel reaction of (–)-drimenol.

Following the facile formation of diene **157**, investigations turned to exploring the elimination reaction. Despite the loss of a stereogenic centre from the elimination, it was thought that stereochemical control in subsequent reactions may still be achieved due to the fixed absolute stereochemistry of the drimane skeleton. The first attempt to synthesise the diene selectively was through acid-catalysed elimination. Unfortunately, treatment of (–)-drimenol with H_2SO_4 in THF resulted in a complicated mixture of products and no spectroscopic evidence of the methylene signals expected for the terminal alkene in the ^{1}H NMR spectrum was observed. A subsequent attempt using PPh_3 and I_2 in CH_2Cl_2 gave a clear colourless oil, which showed in the ^{1}H NMR spectrum ~90% pure diene **157** product without purification. In this case, elimination is favoured over substitution as the I^- is too large to act as an effective nucleophile in this reaction. This was a promising result, however, attempts to repeat and scale up this experiment resulted in formation of the product with much higher levels of undesired and inseparable by-products.



118 **157**

Scheme 4.28 – Dehydration of (–)-drimenol.

An analysis of the X-ray crystal structure of (–)-drimenol provides some evidence towards this by showing the steric bulk around the C-9 position. The reaction is also favoured by the formation of a conjugated diene system. The crystal structure for (–)-drimenol has

been reported previously and showed an unusual Z' value of 3, i.e. 3 molecules in the asymmetric unit of the crystal lattice. The results obtained here are in agreement with those previously published.[296]

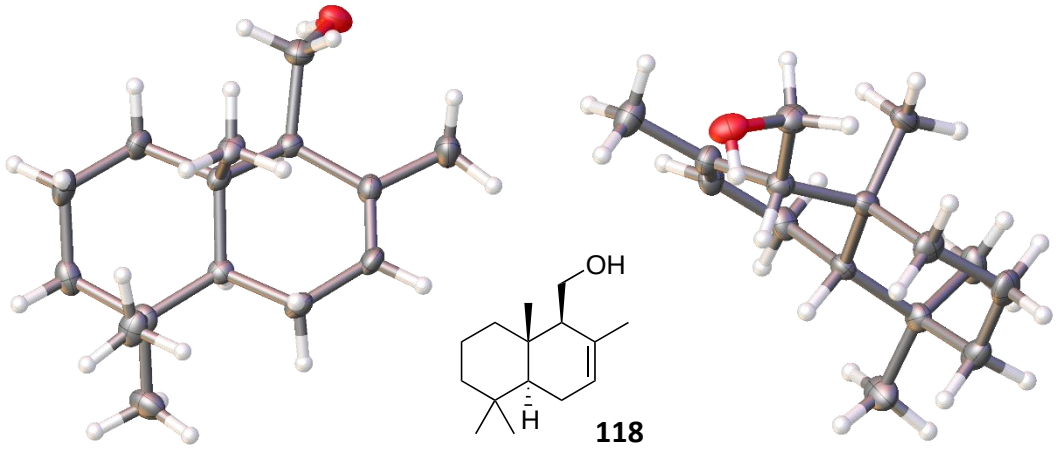
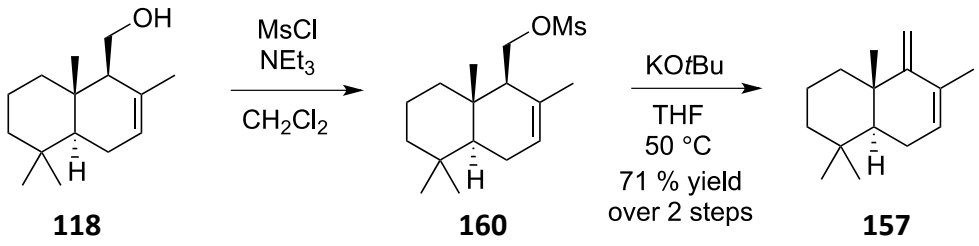


Figure 4.19 – X-ray crystal structure of (–)-drimenol (**118**) showing steric influences around the hydroxymethyl substituent.

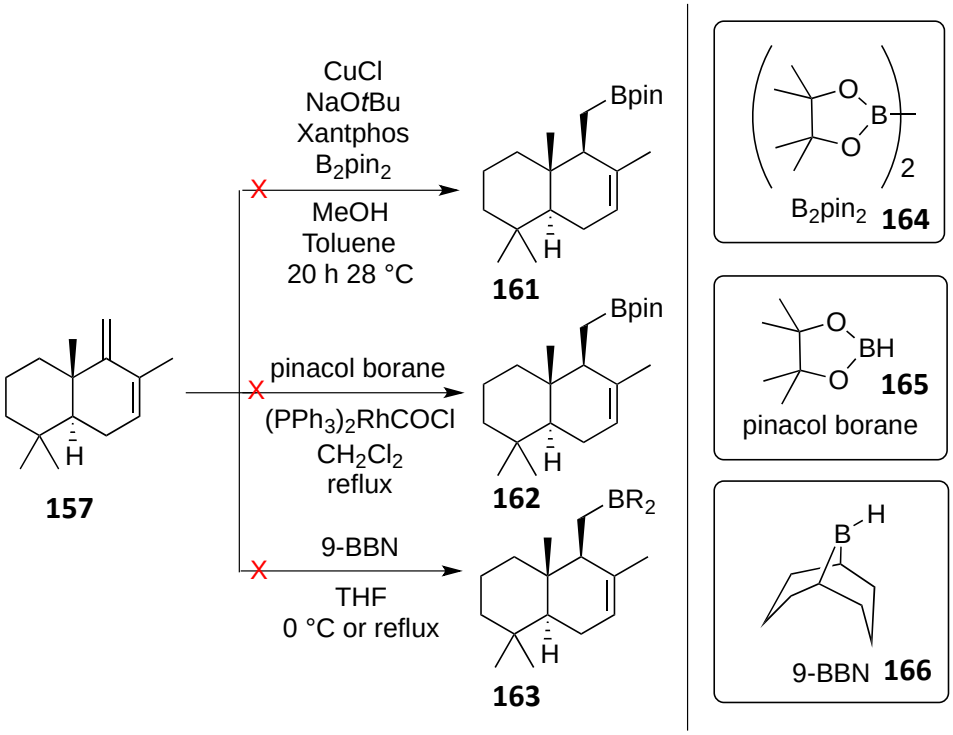
A different leaving group was investigated to attempt to find a substrate that undergoes elimination without significant formation of by-products. (–)-Drimenol was mesylated through reaction with methanesulfonyl chloride. This reaction proceeded within minutes, with high conversion to compound **160**. The mesylate was eliminated without the formation of by-products by the action of potassium *t*-butoxide in THF at 50 °C. In contrast, attempts to form the corresponding tosylate of (–)-drimenol (**118**) were unsuccessful.



Scheme 4.29 – Two–step synthesis of drimendiene **157**.

With drimendiene **157** in hand, the synthesis of a borylated derivative was attempted, with the synthesis of a BF_3K salt **153** in mind. Hydroboration of drimendiene was attempted with pinacolborane (**165**) following conditions published by Reichle and

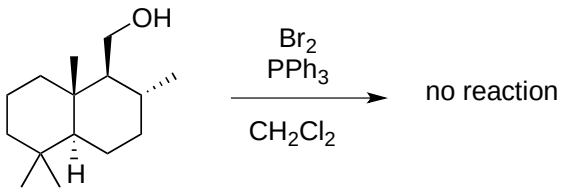
Breit.[297] However, this reaction gave a complicated mixture of products. A second reaction using conditions reported by Tsuji[298] with B_2pin_2 (**164**) as the boron reagent was attempted, and in this case no reaction occurred. Hydroborations were also attempted with 9-BBN (**166**) at high and low temperature, with a complicated mixture of products evident in each case.



Scheme 4.30 – Attempted borylation of drimendiene

4.4.2 Functionalisation of drimanols

Due to the problems with reactivity of drimenol forming a diene system, reactions with the fully saturated analogues were also attempted as model systems where the substrate was not quite so sensitive. Reaction of *anti*–drimanol **119** with PPh_3 and Br_2 resulted in full recovery of starting material, which is an unexpected result for a primary alcohol.



Scheme 4.31 – Attempted bromination of drimanol **119**.

This suggests steric hindrance around the hydroxymethyl centre causing unexpectedly low reactivity. Further evidence for the low reactivity and steric influence of the molecule was observed upon the reaction of the saturated alcohols **119** and **120** with $SOCl_2$ in CH_2Cl_2. These reactions were intended to form the chloro derivative of the alcohol, but these were not observed. Analysis of the reaction by ^1H NMR spectroscopy showed that the reaction is complete almost as soon as the reagents are mixed at room temperature to form two products, which initially were difficult to identify. The ^1H NMR spectra of the reaction mixture prior to purification showed a complex mixture of what appeared to be around 4 different products based on the number of resonances observed between 3.5 and 5 ppm, the region where -CH_2-OH signals are expected. These chemical shifts are higher than the expected shifts for -CH_2-Cl suggesting a lack of chloride incorporation into the molecule.

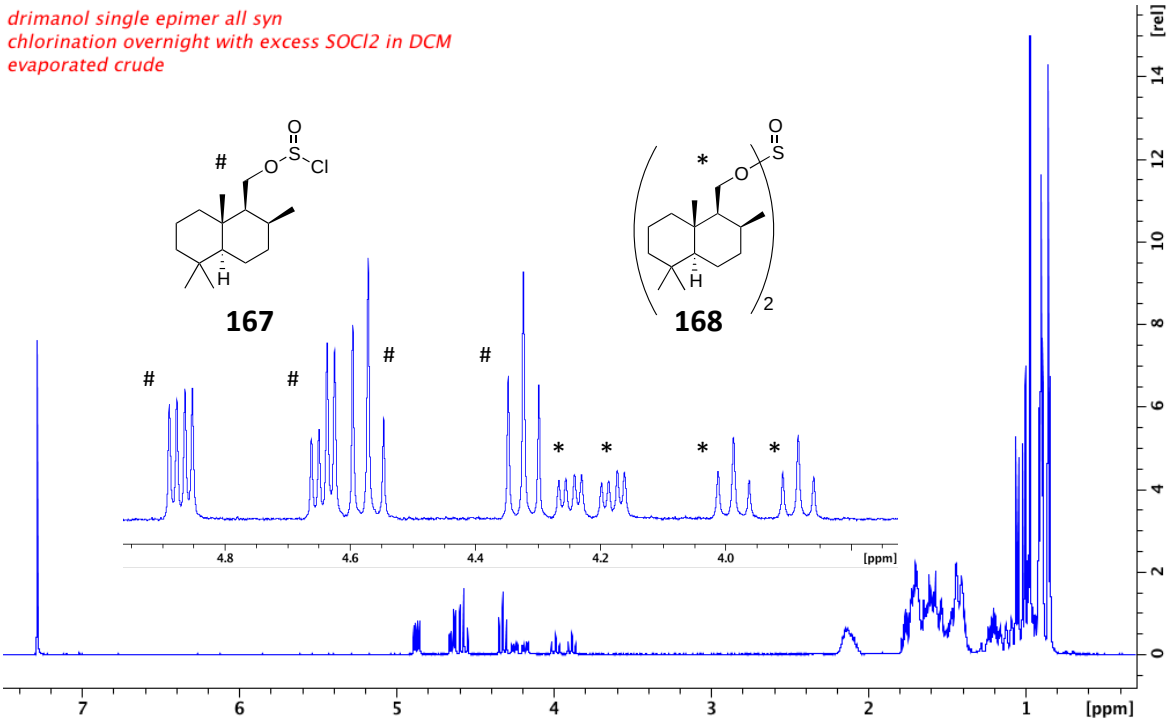


Figure 4.20 – ^1H NMR spectrum of the crude reaction mixture of alcohol **120** with $SOCl_2$.

Purification of the mixture on silica gel gave 2 major fractions. One of these contained the starting material, which was not observed in the ^1H NMR spectrum of the crude reaction mixture. This provided evidence that one of the compounds formed in the reaction was unstable to silica gel chromatography and converted back to the starting material. The other fraction initially appeared to be a mixture of products, as indicated by

a doubling of many of the signals observed in the ^{1}H NMR spectrum. This was also clear in the ^{13}C NMR spectrum which showed most signals either being in pairs, or overlapping causing the signal to be of a greater intensity. Fortuitously, the product readily formed crystals, and the structure of the compound was determined through X-ray crystallography.

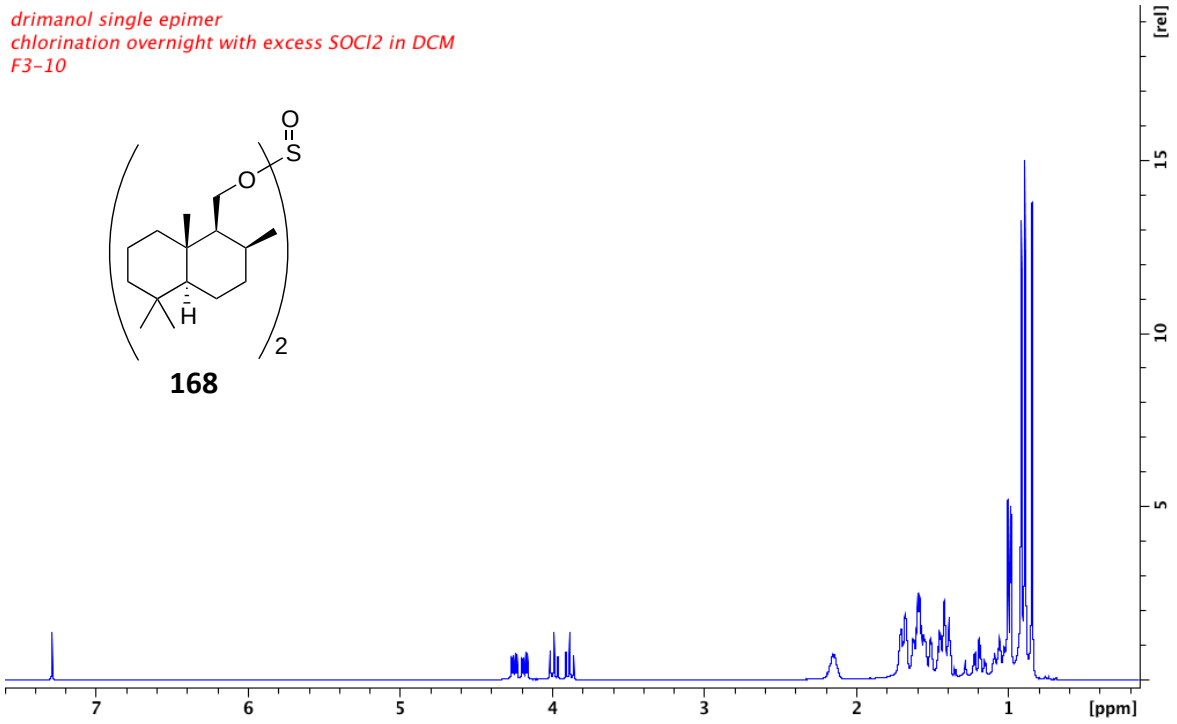


Figure 4.21 – ^{1}H NMR spectrum of the isolated sulfur bridged dimer **168**.

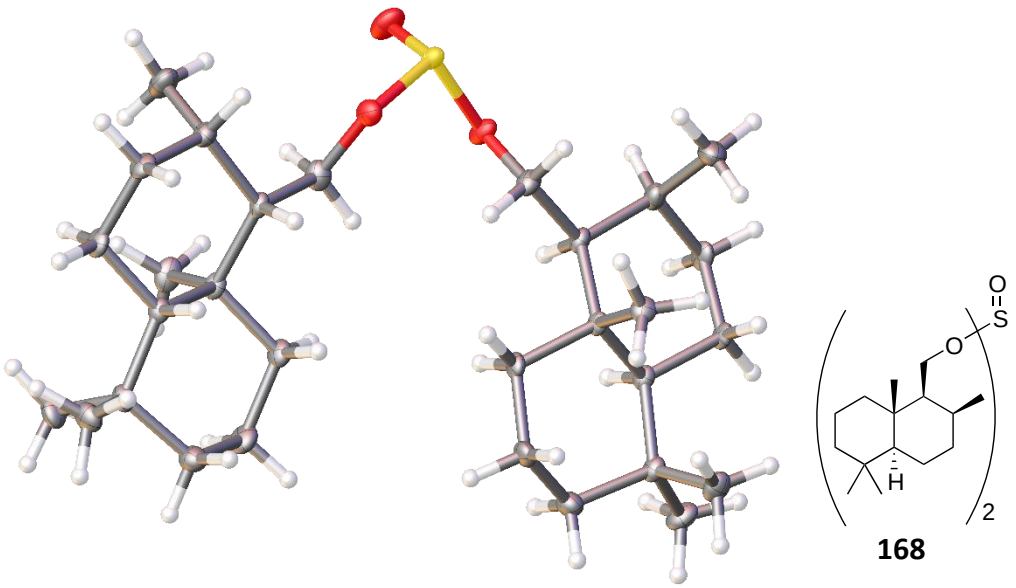
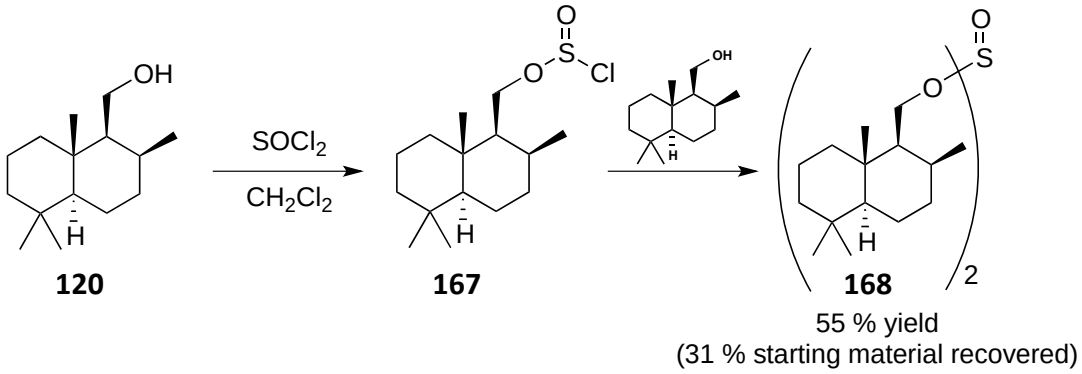


Figure 4.22 – X-ray crystal structure of the sulfur bridged dimer **168**.

Instead of halogenation occurring, the compound was undergoing dimerization through a S=O bridge. This occurs by a stepwise process, where intermediate **167** is formed through nucleophilic attack of the alcohol to the sulphur atom of thionyl chloride. For halogenation, the next step would be the nucleophilic attack of the resulting chloride anion, causing loss of the $OSOCl^-$ group to form the alkyl chloride. In this case, however, the subsequent reaction is a second nucleophilic substitution of the alcohol onto the sulphur, eliminating a second chloride anion and resulting in the dimerised product. This is likely occurring due to the steric hindrance of the drimane skeleton hindering the attack of the chloride anion. This provides further support for the lack of reactivity of this neo-pentyl carbon.

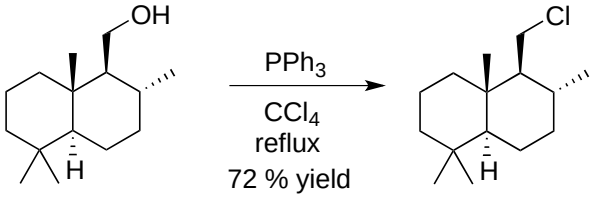


Scheme 4.32 – Stepwise reaction to form the S=O bridged dimer **168**.

This suggested mechanism explains why the reaction is complete immediately upon addition of the alcohol to the $SOCl_2$:CH_2Cl_2 mixture. As the alcohol is added, initially it will react with $SOCl_2$ to form the intermediate. As subsequent alcohol is added, it can either react with $SOCl_2$ or the already formed intermediate to form the dimer. Under these conditions, no reversibility of the intermediate formation is observed which would allow the reaction to proceed to full formation of the dimer. The yield could therefore be optimised if desired by the slow addition of thionyl chloride to a concentrated solution of the alcohol. This unexpected reactivity has been previously observed in the reaction of 10-hydroxycamphor with thionyl chloride.[299]

Other reactions were attempted to halogenate these compounds which were ultimately unsuccessful. *anti*-Drimanol (**119**) was mesylated and subsequently refluxed in acetone with NaI to attempt to install an iodine atom but ultimately only the mesylate was recovered. Reaction with PBr_3 and PCl_5 also yielded complicated mixtures of products.

Chlorination was eventually successful in good yield (72 %) through use of the Appel reaction, refluxing alcohol **119** in CCl_4 with excess PPh_3. The use of CCl_4 is undesirable due to the significant toxicity of this solvent and the expense and difficulty of obtaining it.

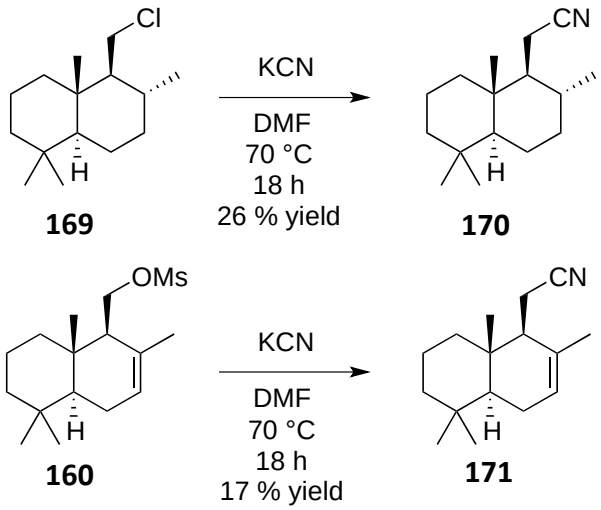


119 **169**

Scheme 4.33 – Halogenation of alcohol **119** through the Appel reaction.

4.4.3 Nitrile Derivatives

Nitrile substitution was attempted on saturated chloride **169** as well as unsaturated mesylate **160** to test if a stronger nucleophile could affect substitution at neopentyl carbon C-12. These reactions allow the extension of the carbon chain by 1 carbon atom, and attach a new functional group, which can undergo reaction with reagents such as Grignard reagents, organolithiums, and cuprates. Nitrile derivatives **170** and **171** were successfully formed through these reactions, in low yields (26 % and 17 %). Analysis of the ^{13}C NMR spectra for these compounds showed the appearance of signals around 120.6 (**170**) and 121.0 ppm (**171**), consistent with the formation of a nitrile derivative. In addition, the IR spectrum for **170** showed a characteristic signal at 2422 cm^{-1}, consistent with the formation of a nitrile.

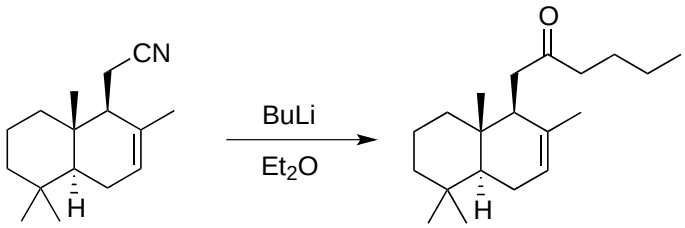


169 **170**

160 **171**

Scheme 4.34 – Formation of nitrile derivatives **170** and **171**.

The substitution of these reagents with $^{-}$CN was slow and required elevated temperature, and resulted in the formation of some of the diene product in the case of drimenyl mesylate (**160**) which is consistent with results from previous reactions. These reactions proceeded in low yields, and were deemed inadequate for larger scale preparation without significant reaction optimisation. Subsequently, unsaturated nitrile derivative **171** was reacted with BuLi in Et_2O to give butyl ketone **172** which was supported by ^{1}H and ^{13}C NMR spectroscopic data of the crude reaction mixture. However, the compound was unstable to chromatographic purification.



171 **172**

Scheme 4.35 – Proposed product of reaction of nitrile **171** with BuLi.

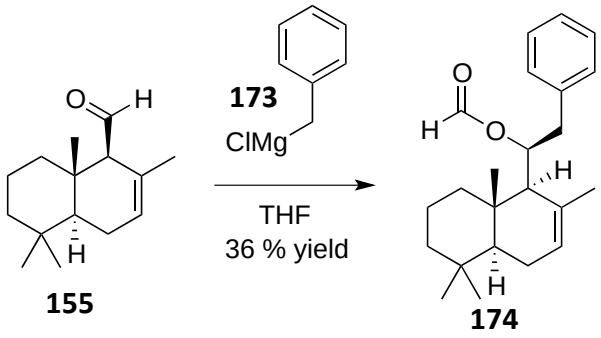
4.4.4 Drimenal Reactivity

Following on from the observed reaction of polygodial towards Grignard reagents, the corresponding aldehyde from (–)-drimenol (**118**), drimenal (**155**), was synthesised for testing with various Grignard reagents. This compound was readily synthesised in good yield (93 %) using Dess-Martin periodinane. This reaction proceeded efficiently at room temperature to furnish the aldehyde **155**. This aldehyde was noted to be unstable in air at ambient temperature, and so was always made immediately prior to reaction or stored at –18 °C under N_2.

The instability of the aldehyde limited the scope of reactivity. Examples of this were the attempted Wadsworth–Emmons olefination with triethyl phosphonoacetate and Wittig reaction with ethyl (triphenylphosphoranylidene)acetate, which yielded no reaction at room temperature, and subsequent heating of the reactions led to the decomposition of the aldehyde.

Drimenal (**155**) was reacted with an excess of benzylmagnesium chloride, which proceeded to furnish formate ester **174** in 36 % yield rather than the secondary alcohol,

which was not the expected product from Grignard addition to aldehyde **155**. The ^{1}H NMR spectrum of the isolated product showed a singlet at 8.02 ppm, and the ^{13}C NMR spectrum showed an extra carbon resonance than expected at 160 ppm. Fortuitously, the product was observed to crystallise in the NMR sample tube, and subsequent analysis by single crystal X-ray diffraction showed that a formate ester had been formed, which was consistent with the aforementioned resonances in the NMR spectra. The NMR spectral data were consistent with the formation of a single diastereomer, and this crystal structure analysis allowed the determination of the absolute stereochemistry for the isomer formed. The source of formate is unknown, but may have been from an incorrectly labelled or contaminated reagent bottle used during workup, or have been the result of an unexpected reaction with CH_2Cl_2.



Scheme 4.36 – Unexpected result from Grignard reaction, formation of formate ester **174.**

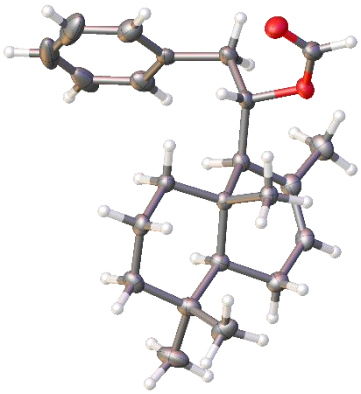
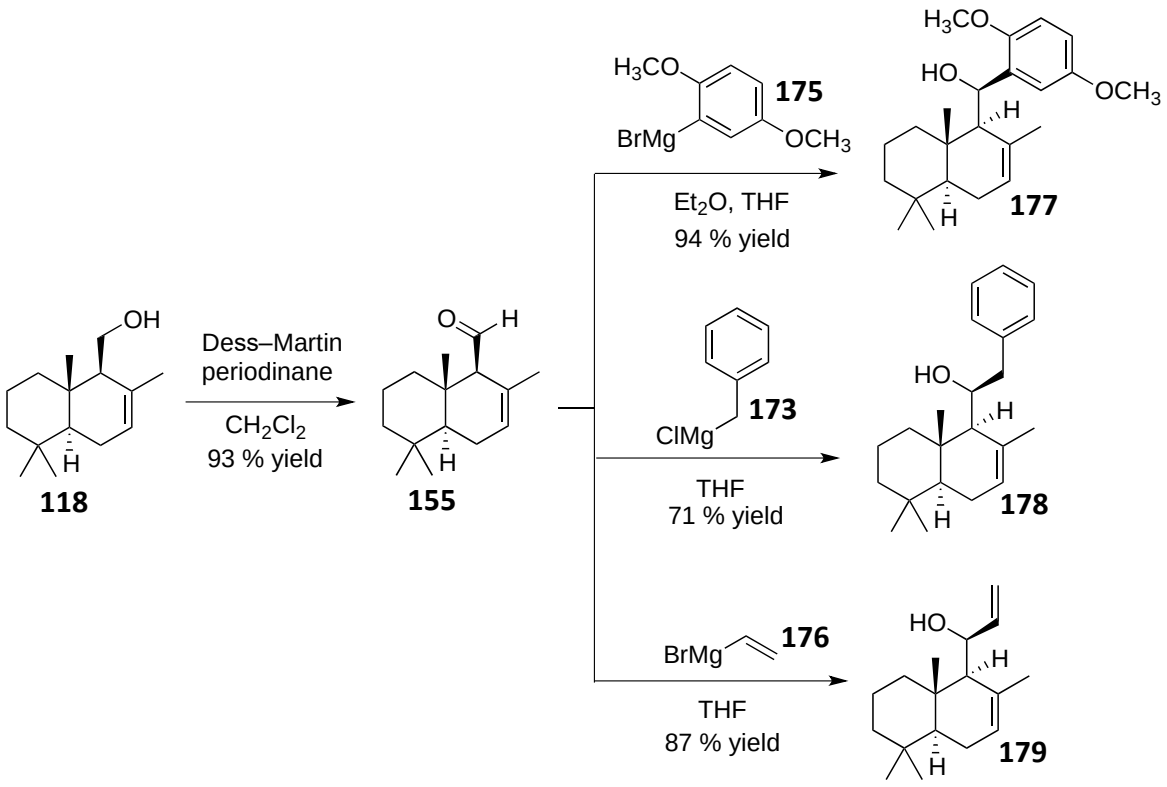


Figure 4.23 – X-ray crystal structure of the formate ester **174** showing absolute stereochemical outcome of the Grignard reaction.

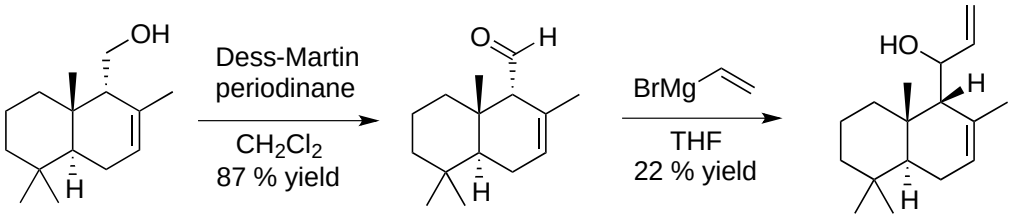
A subsequent repeat of this reaction proceeded to give the expected product **178** in good yield (71 %), supporting that the formate arose from contamination (but was still useful

for determining the absolute stereochemistry). Drimenal (**155**) was then reacted with two further Grignard reagents (**175** and **176**) to confirm the reactivity with a variety of substrates. The reactions proceeded in good yields in each case to form **177** and **179** with high stereospecificity, therefore indicating control of facial addition to this substrate by the axial methyl group (C-15) which can be seen in the X-ray crystal structure of the product in Figure 4.23. This shows that the aldehyde **155** is a potentially useful intermediate for synthesis.



Scheme 4.37 – Synthesis of drimenal **155** followed by Grignard reaction.

The C-8 epimer of drimenal (**180**) was synthesised, and also showed reactivity towards Grignard reagents to form a single diastereomer. This reaction proceeded in a low yield (22 %) but conditions were not optimised. The stereochemistry of the resulting alcohol was not determined for this reaction.

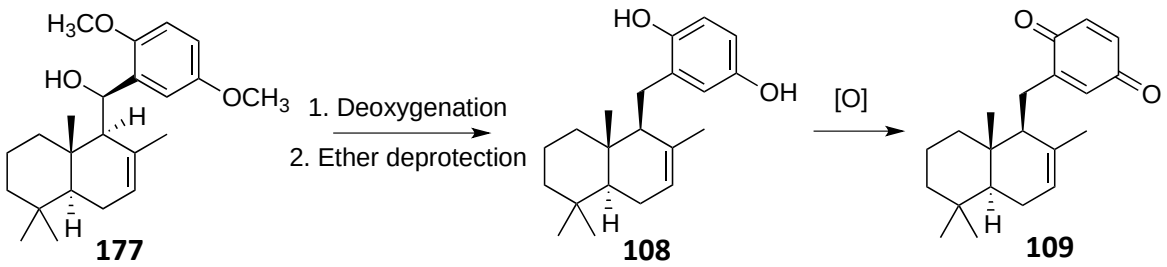


117 **180** **181**

Scheme 4.38 – 9-epidrimenal (**180**) synthesis and subsequent Grignard reaction with vinylMgBr to form **181**. Note - the stereochemistry was not determined for this reaction product.

4.4.5 Future Work from Grignard Reactions

The reaction of drimenal with 2,5-dimethoxyphenylmagnesium bromide to form **177** is of particular significance as it forms a potential intermediate for the synthesis of some meroterpenoid natural products targets such as (–)-isozonarol (**108**) and (–)-isozonarone (**109**). Deoxygenation of the benzylic alcohol, and subsequent ether deprotection would yield (–)-isozonarol (**108**), followed by oxidation to give (–)-isozonarone (**109**). Therefore, compound (**108**) has the potential to be synthesised from polygodial (**12**) in 5 steps.

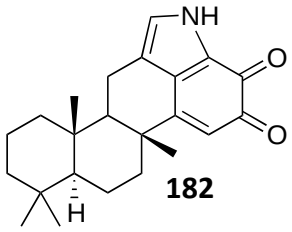


177 **108** **109**

Scheme 4.39 – Natural product targets from the Grignard product **177**, (–)-isozonarol **108** and (–)-isozonarone **109**.

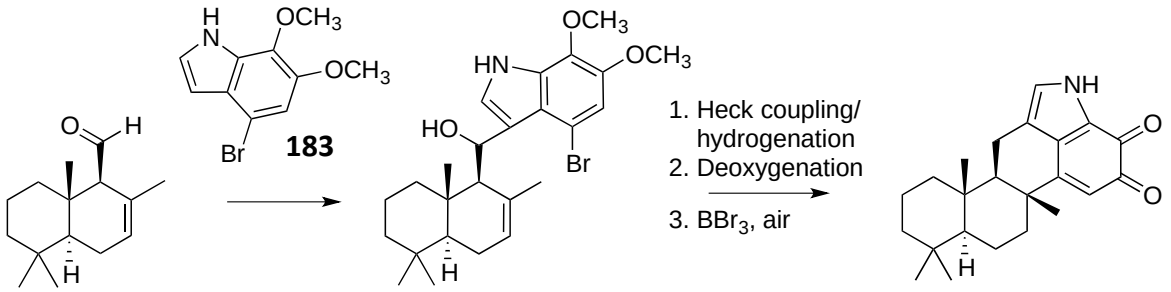
4.4.6 Towards Mycoleptodiscin A

The natural product mycoleptodiscin A (**182**) was discovered and reported in 2013.[300] Subsequently, a 25 step total synthesis with an overall yield of <1 % was published in 2015.[301] This natural product was the target for synthesis from a drimane coupling partner, with an indole derivative as the other half of the molecule in a divergent synthesis. The most promising drimane derivative was the aldehyde drimenal (**155**) as demonstrated through stereospecific reactions with various Grignard reagents. Aldehydes are able to be coupled directly to the 3-position of an indole, catalysed by various Lewis and Brønsted acids.[302-304] These reactions can result in the indole adding twice to the aldehyde, however, it was anticipated that the bulk of the drimane based aldehyde, and control of the amounts of the reagents mono-addition would be favoured.



182

Figure 4.24 – Mycoleptodiscin A

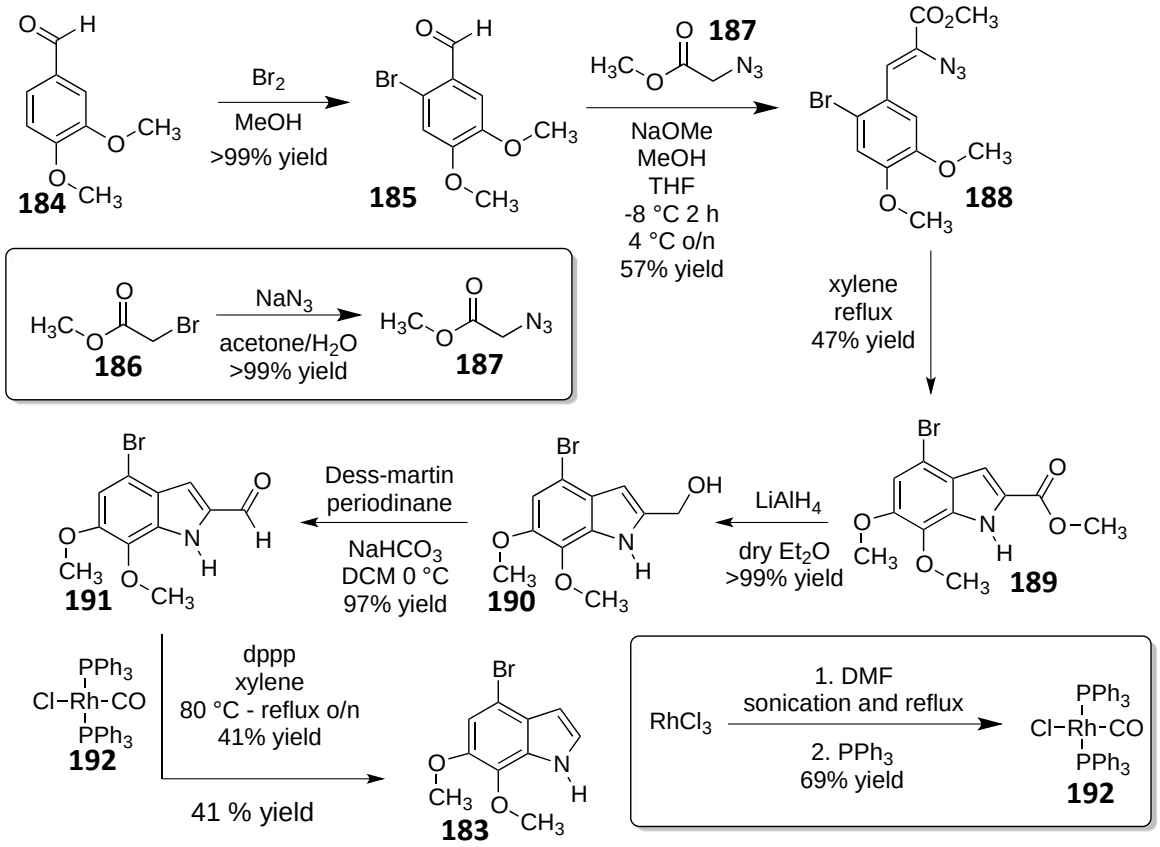


Scheme 4.40 – Proposed synthesis of mycoleptodiscin A (**182**) from polygodial (**12**).

Scheme 4.40 (above) shows the planned route towards **182**, which involves initial addition of indole **183** to aldehyde **155**, followed by intramolecular Heck coupling of the aryl halide to the alkene. Subsequent hydrogenation, deoxygenation, ether deprotection, and aerobic oxidation[301] would form the natural product.

Indole **183** was synthesised in 10.4 % yield over 6 steps, starting from 3,4-dimethoxybenzaldehyde (**184**, veratraldehyde) via a modified literature procedure which

was used for a similar indole derivative.[305] The bromination reaction of **184** proceeded smoothly, with a slight excess of Br_2 in MeOH furnishing the product as a white fluffy solid, which did not require purification. The following step required the synthesis of methyl 2-azidoacetate (**187**) prior to the reaction. Methyl 2-azidoacetate (**187**) was formed quantitatively by reaction of excess sodium azide with methyl 2-bromoacetate (**186**) in a water:acetone mixture and did not require purification. Condensation of methyl 2-azidoacetate (**187**) with 6-bromoveratraldehyde (**185**) proceeded in good yield to give azido ester (**188**). Indole aldehyde **189** was synthesised directly from this substrate through reflux in xylene causing a cyclisation of the azide onto the aromatic ring, with elimination of N_2. This reaction proceeded in moderate yield. The resulting indole **189** then underwent a series of transformations to remove the ester. Reduction to the alcohol with $LiAlH_4$ furnished the alcohol **190** in excellent yield, but this compound was noted to be unstable and required careful handling. The subsequent Dess–Martin periodinane-mediated oxidation required the addition of a base to keep the pH high and avoid acid mediated degradation of the alcohol. This is a divergence from the literature method, which employed MnO_2 for the oxidation.[305] The reaction was carried out at 0 °C to limit thermal degradation, and proceeded in excellent yield to form aldehyde **191**. The final step involved a rhodium catalysed decarbonylation reaction published by Meyer and Kruse.[306] The precatalyst **192** was synthesised from $RhCl_3$ following a literature method.[307] Due to the anhydrous $RhCl_3$ being used, the reaction required a much longer time than reported in combination with sonication to overcome the low solubility of the $RhCl_3$, but this reaction did proceed to give the desired product. The subsequent decarbonylation reaction then required the *in situ* formation of the catalyst from $RhCl(CO)(PPH_3)_2$, with 1,3-bis(diphenylphosphino)propane (dppp), followed by addition of the aldehyde **191**.[306] Overnight reflux, and subsequent work-up and purification afforded indole **183** in moderate yield.



Scheme 4.41 – Synthesis of indole **183**.

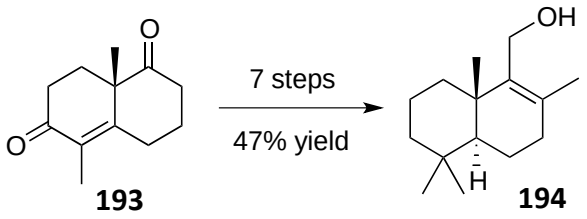
With indole **183** in hand, attempts were made towards the coupling reaction between the aldehyde **155** and indole. Unfortunately, these reactions yielded complicated mixtures of products, presumably due to the instability and limited reactivity of the aldehyde. Due to time constraints within the project, the synthesis of mycoleptodiscin A (**182**) was not pursued further.

4.4.7 Future Work Towards Mycoleptodiscin A

Since this work was undertaken, two more efficient total syntheses have been reported.[308,309] Chandrasikar and co-workers published a synthesis starting from (+)-sclareolide in which they synthesised the product over merely 9 steps in a 19 % overall yield. This synthesis is scalable, providing opportunities for preparation of large quantities of the material, and efficient synthesis of analogues.[308]

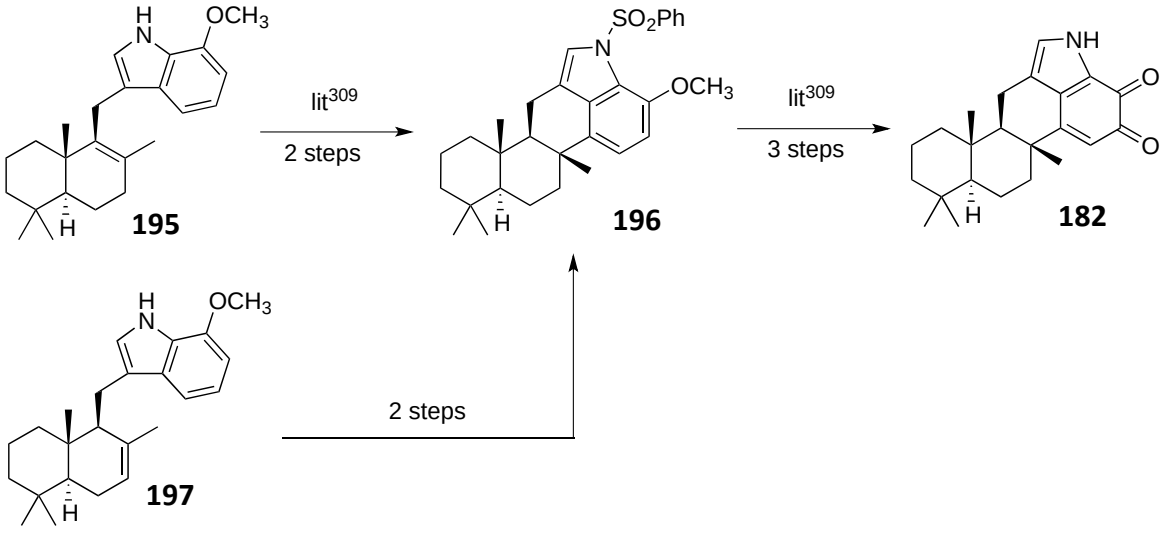
Dethe and co-workers reported a total synthesis using a biomimetic approach. This synthesis makes use of an isomer of (–)-drimenol (**194**) for the drimane portion of the molecule, which was synthesised in 7 steps and 47 % overall yield from the Wieland-

Miescher ketone **193**.[309] This particular route is 13 steps, with a lower overall yield and is therefore not as efficient as Chandrasikar's method. However, due to some of the intermediates, it provides potential opportunity for a formal synthesis of the material efficiently from polygodial (**12**).



Scheme 4.42 – Drimane derivative **194** in Dethe's total synthesis of mycoleptodiscin A.

Instead of using **194** as the drimane source for this synthesis, it is hypothesised that a formal total synthesis of mycoleptodiscin A from (–)-drimenol (**118**) could be achieved, significantly shortening their method. From (–)-drimenol (**118**), common intermediate **196** in their synthesis may be formed through coupling to an indole derivative to form **197**, and subsequent nitrogen protection. If this isomer reacts analogously in the subsequent cyclisation step to form **196**, this would give a total synthesis of mycoleptodiscin A in 6 steps from (–)-drimenol (**118**). The primary difference between these two substrates is that **194** is an allylic alcohol, whereas **118** is not.



Scheme 4.43 – Proposed synthesis of Mycoleptodiscin A (**182**) from (–)-drimenol (**118**).

4.5 Conclusion

Through the investigation of the reactivity of polygodial and related derivatives, a number of natural products and novel non-natural derivatives were synthesised. These include the natural products (+)-euryfuran, drimendiol and (–)-drimenol, as well as non-naturally occurring compounds such as a pyridazine derivative, Wittig adducts, as well as some larger polycyclic systems.

Investigation of various (–)-drimenol derivatives through substitution to form halogen and nitrile derivatives, provided some unexpected difficulties in functionalisation due to the steric hindrance of the neopentyl carbon. However, oxidation to the aldehyde (**155**) provided a scaffold for the synthesis of natural products and derivatives.

Investigation of the Grignard chemistry of drimenal (**155**) provided the most promising results towards the reliable and efficient coupling of the drimane skeleton to various aryl systems in the synthesis of meroterpenoid natural products. Efforts towards the synthesis of mycoleptodiscin A, combined with subsequent literature results have provided a potential synthesis of an interesting natural product in just 6 steps from (–)-drimenol, supporting polygodial as a potentially important scaffold for synthesis.

Chapter 5: Experimental Details

5.1 General Experimental Details

Plant material was ground using a Sunbeam spice/coffee bean grinder. Pressurised hot water extraction (PHWE) was undertaken employing a Breville Espresso Machine Model 800ES and using a Dionex ASE200 Accelerated Solvent Extractor.

Unless otherwise specified, reactions were conducted with magnetic stirring in oven-dried glassware under nitrogen.

Automated gradient flash chromatography was carried out using a Reveleris® X2 Flash Chromatography System using silica gel cartridges. Flash column chromatography was performed using Merck flash grade silica (32-63 μm) according to the general method of Still *et al*.[310]

NMR spectra were recorded on a Bruker NMR spectrometer operating at 400 or 600 MHz (^{1}H) and 100 or 150 MHz (^{13}C). Unless otherwise specified, ^{1}H and ^{13}C NMR spectra were referenced to the solvent signals. The following abbreviations were used to describe ^{1}H, and ^{13}C splitting patterns: s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublets (etc.), q = quartet, quin = quintet, m = multiplet, complex m = complex multiplet where multiple signals overlap.

IR spectra were recorded using a Shimadzu FTIR 8400s instrument on NaCl plates as thin films for all solid and liquid samples.

Microwave irradiation experiments were carried out in a CEM Discover System microwave apparatus (model # 908010).

Mass spectrometry and high-resolution mass spectrometry were performed on a Kratos Concept ISQ mass instrument using electron impact mass spectrometry or by electrospray ionization by direct infusion into an LTQ-Orbitrap XL mass spectrometer using a syringe pump. Accurate mass was measured by “peak matching” at 10000 resolution against perfluorokerosene. Analyses were performed by The Central Science Laboratory at the University of Tasmania.

Optical Rotations were recorded using a Rudolph research analytical Autopol III automatic polarimeter.

Merck silica gel 60 F_{254} aluminium backed sheets were used for analytical thin layer chromatography. TLC plates were visualised under a 254 nm UV lamp and or by treatment with a phosphomolybdic acid (37.5 g), ceric sulfate (7.5 g), sulfuric acid (37.5 mL), water (720 mL) dip or a potassium permanganate dip (3 g $KMnO_4$, 20 g K_2CO_3, 5 mL 5 % aqueous NaOH, 300 mL water), followed by heating.

Pyrethrin samples were analysed using a GC-MS/MS system consisting of a Varian 3800 GC and Bruker 300-MS MS/MS instruments. An Agilent VF-5MS 30 m x 0.25 mm x 0.25 µm capillary column was used with helium as the carrier gas at 1.2 ml/min. Injection volume was 1 µl with split/splitless injector temperature maintained at 290 ^oC, transfer line at 280 ^oC and source temperature 220 ^oC. Oven temperature began at 67 ^oC for 2.5 min, then ramped to 130 ^oC at 40 ^oC/min, then to 150 ^oC at 12 ^oC/min, 250 ^oC at 5.5 ^oC/min, 270 ^oC at 15 ^oC/min and finally to 305 ^oC at 40 ^oC/min, which was maintained for 1.5min. Data was acquired in full scan mode utilising electron impact ionisation over the mass range (m/z) 50 to 500 and processed using Star software (Bruker Star MS Workstation Version 7.0, 2010).

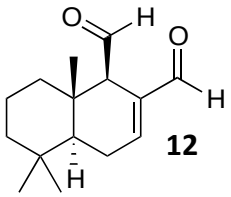
All solvents and reagents were either purchased at high purity suitable for immediate use, or when necessary purified by standard laboratory procedures.

5.2 Chapter 2 Experimental

Polygodial and derivatives

Tasmannia lanceolata leaf material was provided as a finely ground and dried sample by Diemen Pepper (http://www.diemenpepper.com). *T. lanceolata* containing higher polygodial content was provided as fresh leaf material from Essential Oils of Tasmania (http://www.eotasmania.com.au) and dried at 35 °C for 18 h prior to use.

Polygodial – extraction of *T. lanceolata* leaf material from Diemen Pepper (15 g)

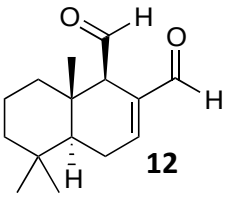


12

Dried, ground *T. lanceolata* leaves (15 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). The extract was concentrated to a volume of ~100 mL under reduced pressure in order to remove EtOH (50 °C water bath temperature). The ensuing mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a viscous, green oil (602 mg). Purification via automated gradient flash chromatography (silica; 0 → 25% EtOAc/hexane) provided the title compound as an off-white solid (130 mg, 0.8% yield w/w). The spectroscopic data obtained was consistent with data reported in the literature.[240]

^{1}H NMR (400 MHz, $CDCl_3$): δ	9.53 (d, *J* = 4.4 Hz, 1H), 9.46 (s, 1H), 7.12 (m, 1H), 2.83 (m, 1H), 2.51 (m, 1H), 2.31 (m, 1H), 1.84 (m, 1H), 1.59–1.45 (complex m, 3H), 1.38 (td, *J* = 12.9, 4.5 Hz, 1H), 1.30–1.19 (complex m, 2H), 0.96 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	202.1, 193.4, 154.4, 138.5, 60.5, 49.2, 41.9, 39.8, 37.1, 33.31, 33.30, 25.4, 22.1, 18.2, 15.5.

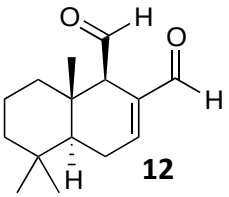
Polygodial – extraction of *T. lanceolata* leaf material from Diemen Pepper (180 g)



12

Dried, ground *T. lanceolata* leaves (15 g) were mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). This was repeated with a further 11 samples of *T. lanceolata* (15 g). The extracts were combined and concentrated to a volume of ~1.2 L under reduced pressure in order to remove EtOH (50 °C water bath temperature). The ensuing mixture was then divided into portions of equal volume (2 × ~600 mL) and each portion was then extracted with CH_2Cl_2 (4 × 100 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a viscous, green oil (6.50 g). Purification via automated gradient flash chromatography (silica; 0 → 25% EtOAc/hexane) provided the title compound as an off-white solid (1.24 g, 0.7% yield w/w)

Polygodial – extraction of *T. lanceolata* leaf material from Essential Oils of Tasmania (30 g)

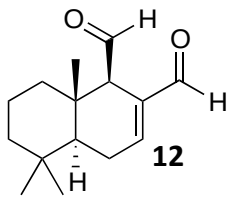


12

Dried *T. lanceolata* leaves (15 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted with 35% EtOH/H_2O (250 mL of a hot solution). This was repeated with another sample of *T. lanceolata* (15 g). The extracts were combined and concentrated to a volume of ~250 mL under reduced pressure in order to remove EtOH (50 °C water bath temperature). The ensuing mixture was extracted with CH_2Cl_2 (5 × 50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a viscous green oil (1.84 g). Purification via automated

gradient flash chromatography (silica; 0 → 25% EtOAc/hexane) provided the title compound as an off-white solid (1.00 g, 3.3% yield w/w).

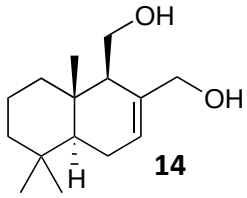
Polygodial (chromatography-free isolation)



12

Dried, ground *Tasmannia lanceolata* leaves (15 g) were mixed with sand (2 g) placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). This was repeated with a further eight samples of *T. lanceolata* to yield 1.80 L of combined extract. This green mixture was extracted with heptane (4 x 200 mL), and the combined organic fractions dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude green oil (4.6 g). Hexane (30 mL) was added to dissolve the extract, which was cooled to 0 °C and seed crystals of polygodial were added. The mixture was allowed to crystallise before being filtered and washed with ice-cold hexane to yield a pale yellow fluffy solid (1.4 g). The filtrate was evaporated to dryness and the crystallisation process repeated to yield further polygodial (1.0 g). Combined yield: 2.4 g, 1.8% w/w (note: the mother liquor contains more polygodial which can be purified by chromatography).

Drimendiol



14

Extraction of *T. lanceolata* leaf material from Diemen Pepper (15 g) was performed as described above to yield 661 mg of the crude extract containing polygodial (**12**). A mixture of this residue in THF (10 mL) was slowly added to a suspension of $LiAlH_4$ (200 mg) in THF (10 mL) maintained at 0 °C. The reaction mixture was then warmed to r.t. and, after 1 h, THF and EtOH were added (5 mL of a 1:1 solution). The insoluble salts were

dissolved by the addition of $KHSO_4$ (80 mL, 2 M aq.). The ensuing mixture was then extracted with CH_2Cl_2 (3 × 50 mL), dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure to provide a dark red oil (632 mg). Purification via flash chromatography (silica; 40 → 50% EtOAc/hexane) provided the title compound as a light-yellow oil (129 mg, 0.8% yield w/w). The spectroscopic data obtained was consistent with data reported in the literature.-[240]

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (m, 1H), 4.30 (d, *J* = 12.3 Hz, 1H), 3.92 (d, *J* = 12.3 Hz, 1H), 3.85 (dd, *J* = 10.9, 1.8 Hz, 1H), 3.61 (dd, *J* = 10.7, 8.31 Hz, 1H), 2.14–1.79 (complex m, 5H), 1.57–1.35 (complex m, 4H), 1.27–1.06 (complex m, 4H), 0.85 (s, 3H), 0.84 (s, 3H), 0.72 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 137.0, 127.1, 67.3, 61.2, 54.5, 49.5, 42.1, 39.4, 35.6, 33.3, 33.0, 23.6, 22.0, 18.9, 14.6.

Maceration of *T. lanceolata* leaf material from Diemen Pepper

Dried, ground *T. lanceolata* leaves (15 g) were added to 35% EtOH/H_2O (200 mL) and the ensuing mixture heated at reflux for 1 h. The mixture was then cooled to r.t. then filtered through Celite™. The resulting green mixture was then extracted with CH_2Cl_2 (2 × 100 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a viscous, dark green oil (180 mg).

Maceration of *T. lanceolata* leaf material from Diemen Pepper

Dried, finely ground *T. lanceolata* leaves (15 g) were stirred in 35% EtOH/H_2O (200 mL) for 24 h. The mixture was then filtered through Celite™ and the ensuing green mixture was then extracted with CH_2Cl_2 (2 × 100 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a viscous, dark green oil (210 mg).

Maceration of *T. lanceolata* leaf material from Diemen Pepper

Dried, finely ground *T. lanceolata* leaves (15 g) were stirred in CH_2Cl_2 (200 mL) for 24 h. The mixture was then filtered through Celite™ and the filtrate was then concentrated under reduced pressure to provide a viscous, dark green residue (1.10 g). Purification via

automated gradient flash chromatography (silica; 0 → 25% EtOAc/hexane) provided the title compound as a dark green oil (164 mg, 1.1% yield w/w).

Dionex ASE200 extraction experiments performed at 500 psi

Dried, ground *T. lanceolata* leaves (~7 g. Diemen Pepper sample) and Celite™ (~3 g) were packed into a Dionex ASE200 extraction cell (22 mL). Experiments were performed using 15, 25, and 35% EtOH/H_2O extraction solutions at 70, 90, and 110 °C, respectively. The following instrument-specific parameters were kept constant across all experiments: - Pressure 500 psi - Preheat time: 0 min - Heat time: 5 min - Static time: 5 min - Flush: 60% - Cycles: 2 - Purge: 90 s - Total extraction time: ~20–25 min - Overall extraction volume: ~35–40 mL

Dionex ASE200 extraction experiments performed at 1500 psi

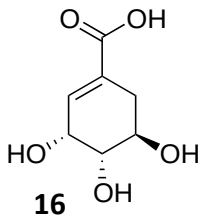
Dried, ground *T. lanceolata* leaves (~7 g. Diemen Pepper sample) and Celite™ (~3 g) were packed into a Dionex ASE200 extraction cell (22 mL). Experiments were performed using 15, 25, and 35% EtOH/H_2O extraction solutions at 70, 90, 110, and 150 °C. The following instrument-specific parameters were kept constant across all extractions: - Pressure 1500 psi - Preheat time: 0 min - Heat time: 5 min (7 min for 150 °C run) - Static time: 5 min - Flush: 60% - Cycles: 2 - Purge: 90 s - Total extraction time: ~20–25 min - Overall extraction volume: ~35–40 mL

Representative work-up procedure for each extract obtained from each Dionex ASE200 extraction experiment

The extract was concentrated to a volume of ~20 mL under reduced pressure to in order to remove EtOH (40 °C water bath temperature). The ensuing mixture was extracted with CH_2Cl_2 (3 × 20 mL) and the organic fractions were combined, dried ($MgSO_4$) and filtered. 1,3,5-Trimethoxybenzene (internal standard, 10.0 mL of a 1.20 M solution in EtOH) was added and the solvent removed under reduced pressure to provide a green residue. The ^{1}H NMR spectrum ($CDCl_3$) of the extract thus obtained enabled the yield of polygodial to be determined. The ratio of polygodial (**12**) to 9-epipolygodial (**80**) was estimated by integration of the two aldehyde signals (9.53 & 9.45 ppm, and 9.84 & 9.40 ppm) respectively relative to the singlet (6.08 ppm) corresponding to 1,3,5-trimethoxybenzene.

Shikimic acid and derivatives

Shikimic acid



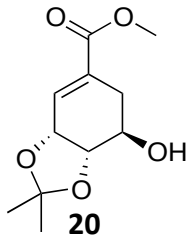
16

Dried Chinese star anise (Illicium verum) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted using 30% EtOH/H_2O (200 mL of a hot solution). This was repeated with another sample of star anise (20 g). The extracts were combined and silica gel (20 g) was added. The ensuing suspension was evaporated to dryness under reduced pressure (50 °C water bath temperature). The ensuing brown solid was then loaded onto a sintered glass funnel and washed with CH_2Cl_2 (150 mL) then EtOAc (150 mL) in order to remove anethole and p-anisaldehyde. These washings were discarded. The resulting solid was then extracted with 10% AcOH/EtOAc (250 mL) and the filtrate was evaporated to dryness. The ensuing solid was then washed with CH_2Cl_2 and then dried under reduced pressure to provide the title compound as an off-white solid (2.21 g, 5.50% yield w/w). The spectroscopic data obtained was consistent with data reported in the literature.[311]

^{1}H NMR (400 MHz, D_2O): δ 6.81 (m, 1H), 4.47 (m, 1H), 4.05 (m, 1H), 3.80 (dd, *J* = 8.2, 4.2 Hz, 1H), 2.77 (ddt, *J* = 18.1, 5.2, 1.4 Hz, 1H), 2.24 (ddt, *J* = 18.2, 6.5, 1.8 Hz, 1H).

^{13}C NMR (100 MHz, D_2O): δ 171.1, 136.8, 130.8, 71.5, 66.9, 66.1, 30.9.

Methyl 3,4-*O*-isopropylideneshikimate



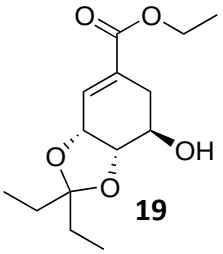
20

Dried Chinese star anise (Illicium verum) pods (15 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H_2O (200 mL of a hot solution). This was repeated with a further 5 samples of star anise (15 g). The extracts were combined and were reduced to 800 mL under reduced pressure (to remove EtOH). The ensuing aqueous phase was washed with CH_2Cl_2 (4 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness under reduced pressure (50 °C water bath temperature) to yield a red/brown sticky solid. MeOH (300 mL) was added to the ensuing residue and then $SOCl_2$ (5 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was a removed under reduced pressure. Acetone (100 mL), 2,2-dimethoxypropane (80 mL), and Amberlyst® 15 acidic ion exchange resin (0.8 g) were added to ensuing dark red/black oil. After 3 h at r.t., the mixture was filtered and evaporated to dryness to provide a dark red/black residue which was then dissolved in CH_2Cl_2 (100 mL) and loaded onto a silica plug (4.5 inch diameter, 1.5 inch depth). 7 fractions were collected: (20 % EtOAc:hexanes (5 x 100 mL), EtOAc (1 x 250 mL, 1 x 100 mL). The title compound was present in the final 2 fractions. After the solvent was removed under reduced pressure, purification via automated gradient flash chromatography (silica; 0 → 70% EtOAc/hexane) provided the title compound as a red oil (4.76 g, equivalent to 4.0 % yield of shikimic acid w/w). The spectroscopic data obtained was consistent with data reported in the literature.[312]

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.91 (m, 1H), 4.74 (m, 1H), 4.08 (m, 1H), 3.89 (m, 1H), 3.76 (s, 3H), 2.78 (dd, *J* = 17.46, 4.7 Hz, 1H), 2.22 (ddt, *J* = 17.4, 8.4, 1.8 Hz, 1H), 2.20 (br s, 1H, OH), 1.44 (s, 3H), 1.39 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 134.2, 130.9, 110.0, 78.2, 72.5, 69.1, 52.4, 29.6, 28.2, 26.0.

Ethyl 3,4-*O*-(3-pentylidene)shikimate



19

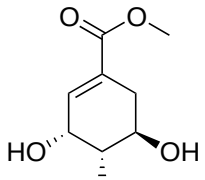
Dried Chinese star anise (Illicium verum) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H_2O (200 mL of a hot solution). This was repeated with a further 9 samples of star anise (20 g). The extracts were combined and were reduced to 800 mL under reduced pressure (to remove EtOH). The ensuing aqueous phase was washed with CH_2Cl_2 (4 x 100 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness under reduced pressure (50 °C water bath temperature) to yield a red/brown sticky solid. EtOH (300 mL) was added to the ensuing residue and then $SOCl_2$ (10 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was a removed under reduced pressure. 3-Pentanone (200 mL), and Amberlyst® 15 acidic ion exchange resin (1.6 g) were added to ensuing dark red/black oil. After 2.5 h at r.t., the mixture was evaporated to dryness to provide a dark red/black residue which was then dissolved in CH_2Cl_2 (100 mL) and loaded onto a silica plug (4.5 inch diameter, 2 inch depth). 7 fractions were collected: (30% EtOAc/hexanes (2 x 150 mL), 50% EtOAc/hexanes (2 x 200 mL), EtOAc (1 x 100 mL). Evaporation of fractions 2–4 provided the title compound as a yellow oil (13.15 g, equivalent to 4.2 % yield of shikimic acid w/w). The spectroscopic data obtained was consistent with data reported in the literature.[313]

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (m, 1H), 4.75 (m, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.11 (m, 1H), 3.91 (m, 1H), 2.77 (dd, *J* = 17.3, 4.6 Hz, 1H), 2.25 (ddt, *J* = 17.3, 8.2, 1.9 Hz, 1H), 1.67 (q, *J* =

7.5 Hz 4H), 1.65 (q, *J* = 7.5 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H), 0.88 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.4, 134.3, 130.6, 113.9, 78.1, 72.5, 69.2, 61.3, 29.9, 29.5, 29.3, 14.4, 8.7, 8.1.

Methyl shikimate



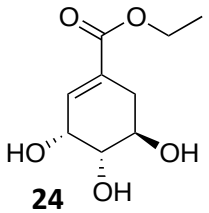
23

Dried Chinese star anise (Illicium verum) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H_2O (200 mL of a hot solution). This was repeated with another sample of star anise (20 g). The extracts were combined and were reduced to 200 mL under reduced pressure (to remove EtOH). The ensuing aqueous phase was washed with CH_2Cl_2 (3 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness under reduced pressure (50 °C water bath temperature) to yield a red/brown sticky solid. MeOH (100 mL) was added to the ensuing residue and then $SOCl_2$ (3 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was a removed under reduced pressure. The resulting dark red/black oil was dissolved in 30% MeOH/EtOAc, filtered through a silica plug eluting with 30% MeOH/EtOAc (300 mL) and the solvent removed under reduced pressure. Purification via automated gradient flash chromatography (silica; 0 → 20% MeOH/EtOAc) provided the title compound as a light-pink solid (2.03 g, equivalent to 4.7 % yield of shikimic acid w/w). The spectroscopic data obtained was consistent with data reported in the literature.[80]

^{1}H NMR (400 MHz, D_2O): δ 6.72 (m, 1H), 4.35 (m, 1H), 3.93 (m, 1H), 3.68 (m, 1H), 3.66 (s, 3H), 2.65 (dd, *J* = 18.2, 5.0 Hz, 1H), 2.14 (dd, *J* = 18.3, 6.3 Hz, 1H).

^{13}C NMR (100 MHz, DMSO-d6): δ 166.7, 139.8, 127.3, 70.0, 66.8, 65.4, 51.5, 29.6.

Ethyl shikimate



24

Dried Chinese star anise (Illicium verum) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H_2O (200 mL of a hot solution). This was repeated with another sample of star anise (20 g). The extracts were combined and were reduced to 200 mL under reduced pressure (to remove EtOH). The ensuing aqueous phase was washed with CH_2Cl_2 (3 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness under reduced pressure (50 °C water bath temperature) to yield a red/brown sticky solid. EtOH (100 mL) was added to the ensuing residue and then $SOCl_2$ (3 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was a removed under reduced pressure. The resulting dark red/black oil was dissolved in 30% MeOH/EtOAc, filtered through a silica plug eluting with 30% MeOH/EtOAc (300 mL) and the solvent removed under reduced pressure. Purification via automated gradient flash chromatography (silica; 0 → 10% MeOH/EtOAc) provided the title compound as a light-brown solid (2.49 g, equivalent to 5.4 % yield of shikimic acid w/w). The spectroscopic data obtained was consistent with data reported in the literature.[81]

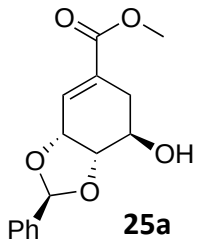
^{1}H NMR (400 MHz, DMSO-d6): δ 6.62 (m, 1H), 4.82 (m, 2H, OH), 4.61 (d, *J* = 4.3 Hz, 1H, OH), 4.23 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.86 (m, 1H), 3.57 (m, 1H), 2.43 (m, 1H), 2.05 (m, 1H), 1.22 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d6): δ 166.7, 140.0, 128.1, 70.5, 67.3, 65.9, 60.5, 30.1, 14.6.

Methyl 3,4-*O*-benzylideneshikimate (mixture of diastereomers)

Dried Chinese star anise (*Illicium verum*) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H_2O (200 mL of a hot solution). This was repeated with another sample of star anise (20 g). The extracts were combined and were reduced to 200 mL under reduced pressure (to remove EtOH). The ensuing aqueous phase was washed with CH_2Cl_2 (3 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness under reduced pressure (50 °C water bath temperature) to yield a red/brown sticky solid. MeOH (100 mL) was added to the ensuing residue and then $SOCl_2$ (3 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was a removed under reduced pressure. THF (100 mL), benzaldehyde (3 mL), and Amberlyst® 15 acidic ion exchange resin (1.6 g) were added to ensuing black oil. After 2 h at r.t., the mixture was evaporated to dryness to provide a black residue which was then and loaded onto a silica plug (4.5 inch diameter, 1.5 inch depth). 4 fractions were collected: 50% EtOAc/hexanes (3 x 150 mL), 70 % EtOAc/hexanes (1 x 150 mL). The title compound was present in fractions 2–4. After the solvent was removed under reduced pressure, purification via automated gradient flash chromatography (silica; 0 → 100% EtOAc/hexane) provided the title compound as a mixture of diastereomers as a yellow oil (2.34 g, equivalent to 3.5 % yield of shikimic acid w/w). These diastereomers were partially separable by chromatography. The spectroscopic data obtained was consistent with data reported in the literature.[314]

(S)-Methyl 3,4-*O*-benzylideneshikimate

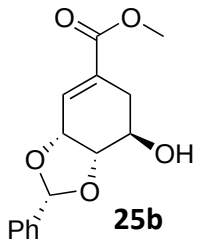


25a

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (m, 2H), 7.38 (m, 3H), 6.93 (m, 1H), 5.97 (s, 1H), 4.87 (m, 1H), 4.34 (m, 1H), 4.14 (m, 1H), 3.79 (s, 3H), 2.84 (dd, *J* = 17.3, 4.5 Hz, 1H), 2.34 (ddt, *J* = 17.5, 7.2, 1.8 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 137.9, 133.7, 131.3, 129.5, 128.6, 126.5, 102.9, 78.1, 72.7, 67.3, 52.4, 29.1.

(R)-Methyl 3,4-*O*-benzylideneshikimate



25b

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (m, 2H), 7.37 (m, 3H), 7.00 (m, 1H), 5.94 (s, 1H), 4.80 (m, 1H), 4.18 (m, 1H), 3.93 (m, 1H), 3.78 (s, 3H), 2.83 (dd, *J* = 17.4, 4.8 Hz, 1H), 2.27 (ddt, *J* = 17.5, 9.0, 1.9 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 136.6, 133.0, 131.7, 129.9, 128.7, 127.0, 104.6, 78.8, 74.2, 69.3, 52.4, 29.7.

***Correa* spp.**

***Correa reflexa* extraction**

Correa reflexa leaves were dried in an oven overnight at 40 °C. Finely ground leaves (10 g) were mixed with sand (2 g), placed in to the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). This was repeated with a further 9 samples of *C. reflexa* (10 g). The extracts were combined and concentrated to a volume of ~1 L under reduced pressure in order to remove EtOH (50 °C water bath temperature). The ensuing mixture was extracted with EtOAc (1 x 200 mL, 5 x 100 mL), the extracts combined, dried ($MgSO_4$), filtered, and the solvent removed to yield a green oil (3.9 g). Purification via automated gradient flash chromatography (silica; 0 → 100% EtOAc/hexane) provided seselin (**29**) as an off-white solid (1.00 g, 1.00% yield w/w) and (+)-epoxysuberosin (**30**) as an off–white solid (1.15 g, 1.15% yield w/w).

***Correa alba* extraction**

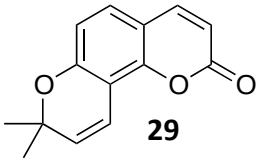
Correa alba leaves were dried in an oven overnight at 40 °C. Finely ground leaves (10 g) were mixed with sand (2 g), placed in to the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). This was repeated with a further 2 samples of *C. alba* (10 g). The extracts were combined and concentrated to a volume of ~300 mL under reduced pressure in order to remove EtOH (55 °C water bath temperature). The ensuing mixture was extracted with EtOAc (3 x 100 mL), the extracts combined, dried ($MgSO_4$), filtered, and the solvent removed to yield a green oil (0.8 g). Purification via automated gradient flash chromatography (silica; 0 → 15% EtOAc/CH_2Cl_2) provided alosin (**31**) as a white solid (268 mg, 0.89% yield w/w), and (–)-meranzin (**32**) as an off-white solid (94 mg, 0.31% yield w/w).

***Correa backhouseana* extraction**

Correa backhouseana leaves were dried in an oven overnight at 40 °C. Finely ground leaves (10 g) were mixed with sand (2 g), placed in to the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). This was repeated with a further 9 samples of *C. backhouseana* (10 g). The extracts were combined and concentrated to a volume of ~1 L under reduced pressure in order to remove EtOH (55 °C water bath temperature). The ensuing mixture was extracted with EtOAc (4 x 100 mL), the extracts combined, dried ($MgSO_4$), filtered, and the solvent removed to yield a green oil (1.1 g). Purification via automated gradient flash chromatography (silica; 0 → 15% EtOAc/CH_2Cl_2) provided alosin (**31**) as a white solid (65 mg, 0.065% yield w/w) and meranzin (**32**) as an off–white solid (50 mg, 0.050% yield w/w). The heraclenin fractions contained impurities, further purification via automated gradient flash chromatography (silica; 0 → 8 % EtOAc/CH_2Cl_2) provided heraclenin (**33**) as a pale yellow oil (41 mg, 0.041% yield w/w).

Seselin

The spectroscopic data obtained was consistent with data reported in the literature.[140]

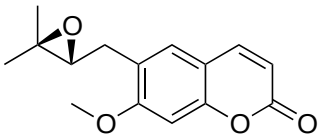


29

^{1}H NMR (600 MHz, $CDCl_3$): δ	7.59 (d, *J* = 9.5 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 6.87 (dd, *J* = 10.1, 0.6 Hz, 1H), 6.71 (dd, *J* = 8.5 and 0.6 Hz, 1H), 6.21 (d, *J* = 9.5 Hz, 1H), 5.72 (d, *J* = 10.1 Hz, 1H), 1.47 (s, 6H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	161.0, 156.5, 150.3, 144.0, 130.9, 127.9, 115.1, 113.7, 112.75, 112.73, 109.4, 77.8, 28.3.

(+)-Epoxysuberosin

The spectroscopic data obtained was consistent with data reported in the literature.[315,316]

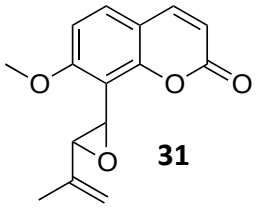


30

^{1}H NMR (600 MHz, $CDCl_3$): δ	7.62 (d, *J* = 9.4 Hz, 1H), 7.30 (s, 1H), 6.80 (s, 1H), 6.24 (d, *J* = 9.4 Hz, 1H), 3.90 (s, 3H), 2.99 (m, 2H), 2.74 (m, 1H), 1.39 (s, 3H), 1.33 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	161.4, 160.8, 155.1, 143.6, 128.6, 124.3, 113.3, 112.2, 98.9, 63.4, 59.0, 56.1, 29.3, 24.9, 19.1.
$[\alpha]_D^{28}$	+19 (c 2.6, $CHCl_3$), lit. $[\alpha]_D$ +34 (c 0.07, $CHCl_3$)[316]

Alosin

The spectroscopic data obtained was consistent with data reported in the literature.[317]

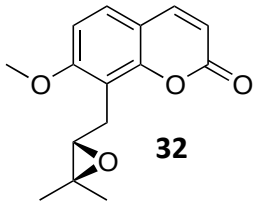


31

^{1}H NMR (600 MHz, $CDCl_3$): δ	7.61 (d, *J* = 9.5 Hz, 1H), 7.41 (d, *J* = 8.6 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 1H), 6.26 (d, *J* = 9.5 Hz, 1H), 5.29 (m, 1H), 5.07 (m, 1H), 3.98 (d, *J* = 2.4 Hz, 1H), 3.96 (s, 3H), 3.91 (d, *J* = 2.4 Hz, 1H), 1.87 (m, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	162.1, 160.4, 154.1, 143.5, 141.5, 129.1, 113.63, 113.61, 113.0, 112.8, 107.7, 60.8, 56.5, 51.9, 17.6.
$[\alpha]_D^{28}$	13 (c 1.27, $CHCl_3$)*

(–)-Meranzin

The spectroscopic data obtained was consistent with data reported in the literature.[318]



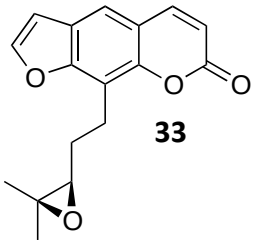
32

^{1}H NMR (600 MHz, $CDCl_3$): δ	7.63 (d, *J* = 9.5 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 6.86 (d, *J* = 8.6 Hz, 1H), 6.24 (d, *J* = 9.5 Hz, 1H), 3.93 (s, 3H), 3.20 (m, 1H), 3.11 (m, 2H), 1.49 (s, 3H), 1.28 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	161.1, 160.9, 153.6, 143.8, 127.3, 114.4, 113.3, 113.1, 107.5, 63.1, 59.4, 56.3, 24.9, 22.6, 19.3.
$[\alpha]_D^{28}$	+43 (c 2.4, $CHCl_3$), lit. $[\alpha]_D$ −46 (c 0.063, $CHCl_3$)[318]

* See Deans *et al.* (2017) for discussion of optical rotations for coumarins from *Correa* spp.[46]

Heraclenin

The spectroscopic data obtained was consistent with data reported in the literature.[157]



33

^{1}H NMR (600 MHz, $CDCl_3$): δ	7.77 (d, *J* = 9.6 Hz, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.40 (s, 1H), 6.82 (d, *J* = 2.0 Hz, 1H), 6.37 (d, *J* = 9.6 Hz, 1H), 4.58 (m, 2H), 3.32 (t, *J* = 5.6 Hz, 1H), 1.34 (s, 3H), 1.28 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	160.4, 148.5, 147.0, 144.4, 143.8, 131.6, 126.1, 116.7, 115.0, 114.0, 107.0, 72.6, 61.5, 58.3, 24.7, 19.0.
$[\alpha]_D^{28}$	+1.8 (c 1.3, $CHCl_3$), –6.5 (c 1.3, C_5H_5N)*

* See Deans *et al.* (2017) for discussion of optical rotations for coumarins from *Correa* spp.[46]

5.3 Chapter 3 Experimental

Crofton weed (*Eupatorium adenophorum*) extraction – first extraction

Dried, ground *E. adenophorum* leaves (10 g) were mixed with sand (5 g) placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200mL of a hot solution). This process was repeated with a further 2 x 10 g of leaf material. This green mixture was evaporated to ~half volume at 50 °C under reduced pressure before being extracted with heptane (3 x 100 mL), and the combined organic fractions dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil (506 mg). Purification via multiple automated gradient flash column chromatography runs yielded the compounds described in Chapter 3. The residual aqueous fraction was then extracted with EtOAc (2 x 50 mL), dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude dark green flaky solid (751 mg). Purification via automated gradient flash column chromatography (silica; 0 → 60 % EtOAc/hexane) in normal phase provided compounds **56** and **57** as a mixture (120 mg, 0.40 % yield w/w). Further purification of a small portion via automated gradient flash column chromatography in reversed-phase (C_{18} silica; 0 → 30% water:CH_3CN) yielded the compounds isolated for characterisation.

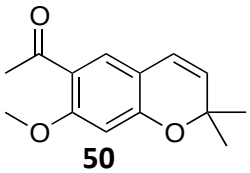
Crofton weed (*Eupatorium adenophorum*) extraction – second extraction

Dried, ground *E. adenophorum* leaves (10.0 g) were mixed with sand (5 g) placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200mL of a hot solution). This process was repeated with a further 2 x 10.0 g and 1 x 7.5 g of leaf material. This combined green mixture (800 mL) was evaporated to ~half volume at 50 °C under reduced pressure before being extracted with heptane (3 x 100 mL), and the combined organic fractions dried over $MgSO_4$, filtered and evaporated to dryness to yield 900 mg of a crude pale green oil. Purification via automated gradient flash column chromatography (silica; 0 → 25 % EtOAc/hexane) provided compounds as pure compounds or mixtures as appropriate.

Compound	Yield (mg)	Yield (% w/w)
50	4	0.01
34, **46**	77	0.21
37, **51**, **35**	428	1.14
38	43	0.11
Total (excl **50**)	548	1.46

1-(7-methoxy-2,2-dimethyl-2*H*-chromen-6-yl)ethanone

The spectroscopic data obtained was consistent with data reported in the literature.[183]



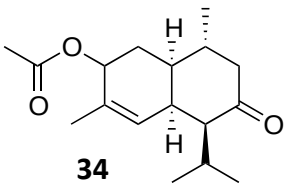
50

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.53 (s, 1H), 6.38 (s, 1H), 6.30 (d, *J* = 9.9 Hz, 1H), 5.52 (d, *J* = 9.9 Hz, 1h), 3.87 (s, 3H), 2.56 (s, 3H), 1.44 (s, 6H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 197.8, 161.3, 158.6, 129.2, 128.5, 121.5, 120.8, 114.1, 99.8, 77.8, 55.8, 32.1, 28.6.

(4a*R*,5*S*,8*R*,8a*S*)-5-isopropyl-3,8-dimethyl-6-oxo-1,2,4a,5,6,7,8,8a-octahydronaphthalen-2-yl acetate

The spectroscopic data obtained was consistent with data reported in the literature.[165]



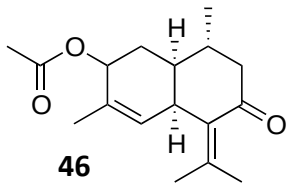
34

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.34 (s, 1H), 5.18 (d, *J* = 4.6 Hz, 1 H), 2.72 (m, 1H) 2.30 (m, 1H), 2.23 (m, 1H), 2.15 (d, *J* = 8.7 Hz, 2H), 2.07–1.99 (complex m, 4H), 1.91–1.83 (complex m, 3H), 1.63 (s, 3H), 1.02 (d, *J* = 6.5 Hz, 3H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 214.4, 170.9, 132.5, 130.4, 69.4, 64.5, 46.7, 41.7, 35.3, 32.2, 30.7, 28.5, 21.4, 21.3, 20.5, 20.4, 20.2.

(4a*R*,8*R*,8a*S*)-3,8-dimethyl-6-oxo-5-(propan-2-ylidene)-1,2,4a,5,6,7,8,8a-octahydronaphthalen-2-yl acetate

The spectroscopic data obtained was consistent with data reported in the literature.[168]



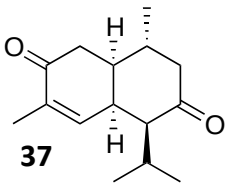
46

^{1}H NMR (600 MHz, $CDCl_3$): δ 5.24 (s, 1H), 5.18 (d, *J* = 4.9 Hz, 1H), 3.57 (m, 1H), 2.39 (m, 1H), 2.34 (m, 1H), 2.28 (m, 1H), 2.08–2.02 (complex m, 4H)**,** 1.97 (s, 3H), 1.91 (dt, *J* = 15.2, 4.6 Hz, 1H), 1.81 (s, 3H), 1.77 (m, 1H), 1.63 (s, 3H), 0.96 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 204.3, 170.9, 141.7, 135.8, 131.9, 129.4, 68.3, 51.2, 42.1, 38.5, 30.4, 29.5, 23.0, 21.6, 21.2, 20.1, 20.0.

(1*S*,4*R*,4a*S*,8a*R*)-1-isopropyl-4,7-dimethyl-1,3,4,4a,5,8a-hexahydronaphthalene-2,6-dione

The spectroscopic data obtained was consistent with data reported in the literature.[164]



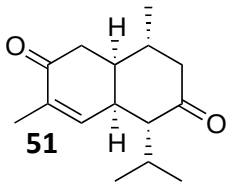
37

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.38 (m, 1H), 3.37 (m, 1H), 2.76 (dd, *J* = 16.6, 2.5 Hz, 1H), 2.57 (dd, *J* = 16.8, 4.6 Hz, 1H), 2.32 (dd, *J* = 10.5, 5.5 Hz, 1H), 2.29–2.22 (complex m, 2H), 2.19 (m, 1H), 2.12 (t, *J* = 12.2 Hz, 1H), 1.96 (m, 1H), 1.70 (m, 3H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.93 (d, *J* = 6.2 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 210.8, 198.2, 141.5, 136.8, 60.3, 50.4, 45.2, 43.8, 41.8, 33.3, 23.3, 22.6, 20.1, 19.1, 15.9.

(1*R*,4*R*,4a*S*,8a*R*)-1-isopropyl-4,7-dimethyl-1,3,4,4a,5,8a-hexahydronaphthalene-2,6-dione

The spectroscopic data obtained was consistent with data reported in the literature.[164]



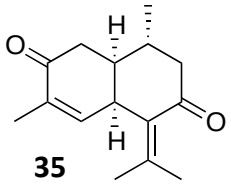
51

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.34 (s, 1H), 3.21 (s, 1H), 2.79 (dd, *J* = 16.5, 3.0 Hz, 1H), 2.52 (dd, *J* = 16.6, 4.3 Hz, 1H), 2.27 (m, 1H), 2.19 (m, 2H), 2.11–1.96 (complex m, 3H), 1.73 (s, 3H), 1.07 (d, *J* = 5.6 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 5.8 Hz, 3H)

^{13}C NMR (150 MHz, $CDCl_3$): δ 213.1, 198.3, 147.1, 136.4, 64.2, 46.1, 42.4, 41.3, 39.5, 32.0, 28.4, 21.1, 20.5, 20.2, 15.7.

(4*R*,4a*S*,8a*R*)-4,7-dimethyl-1-(propan-2-ylidene)-1,3,4,4a,5,8a-hexahydronaphthalene-2,6-dione

The spectroscopic data obtained was consistent with data reported in the literature.[168]



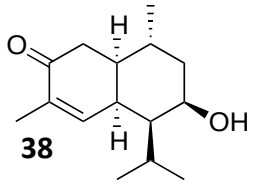
35

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.22 (m, 1H), 4.06 (sept, *J* = 2.4 Hz, 1H), 2.81 (dd, *J* = 16.3, 2.7 Hz, 1H), 2.62 (dd, *J* = 16.3, 4.5 Hz, 1H), 2.44 (d, *J* = 10.4 Hz, 1H), 2.19 (m, 1H), 2.14–2.05 (complex m, 2H), 2.02 (s, 3H), 1.89 (s, 3H), 1.73 (dd, *J* = 2.7, 1.3 Hz, 3H), 0.98 (d, *J* = 5.9 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 203.0, 197.9, 146.5, 143.9, 136.2, 134.5, 50.8, 43.9, 43.1, 41.7, 29.4, 23.3, 22.2, 19.8, 15.6.

(4a*R*,5*S*,6*R*,8*R*,8a*S*)-6-hydroxy-5-isopropyl-3,8-dimethyl-4a,5,6,7,8,8a-hexahydronaphthalen-2(1*H*)-one

The spectroscopic data obtained was consistent with data reported in the literature.[164]

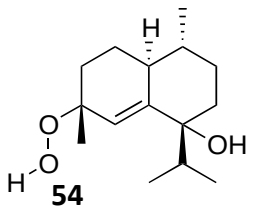


38

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.81 (s, 1H), 4.14 (m, 1H), 2.95 (m, 1H), 2.77 (dd, *J* = 16.4, 2.5 Hz, 1H), 2.45 (dd, *J* = 16.3, 4.7 Hz, 1H), 2.10 (m, 1H), 1.87–1.77 (complex m, 1H), 1.75 (m, 3H), 1.66 (m, 1H), 1.22 (m, 1H), 1.14 (ddd, *J* = 10.6, 5.1, 1.9 Hz, 1H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 199.6, 146.4, 133.5, 67.4, 51.8, 45.7, 43.0, 42.8, 37.5, 25.3, 23.1, 21.1, 20.7, 19.3, 16.1.

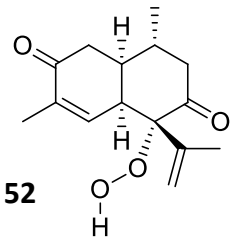
(1R,4R,4aS,7R)-7-hydroperoxy-1-isopropyl-4,7-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-1-ol



54

crystal only - data presented in X-ray crystallography section

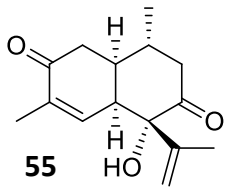
(1*S*,4*R*,4a*S*,8a*R*)-1-hydroperoxy-4,7-dimethyl-1-(prop-1-en-2-yl)-1,3,4,4a,5,8a-hexahydronaphthalene-2,6-dione



52

HRMS	Found $[M+Na]^+$ 287.1254, $C_{15}H_{20}O_4Na^+$ requires M^+ 287.1254.
ν_{max} (NaCl):	3308 (broad O-OH), 2963, 2926, 2876, 1724 (C=O), 1674 (C=O), 1452, 1373, 1207, 1113 1090, 1024, 976, 924, 754 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	8.18 (s, 1H, O-OH), 6.34 (s, 1H), 5.61 (s, 1H), 5.52 (m, 1H), 3.38 (sept, *J* = 2.5 Hz, 1H), 2.86 (t, *J* = 12.2 Hz, 1H), 2.74 (dd, *J* = 16.6, 3.6 Hz, 1H), 2.55 (m, 1H), 2.50 (dd, *J* = 16.6, 4.6 Hz, 1H), 2.28 (dd, *J* = 12.6, 5.1 Hz, 1H), 2.04 (m, 1H), 1.87 (s, 3H), 1.75 (dd, *J* = 2.6, 1.5 Hz, 3H), 1.06 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$):	208.4, 198.0, 139.5, 138.0, 137.7, 121.7, 91.9, 46.4, 45.6, 40.9, 39.6, 33.2, 20.1, 19.9, 16.0
$[\alpha]_D^{22}$	+82 (c 0.6, $CHCl_3$)

(1*S*,4*R*,4a*S*,8a*R*)-1-hydroxy-4,7-dimethyl-1-(prop-1-en-2-yl)-1,3,4,4a,5,8a-hexahydronaphthalene-2,6-dione



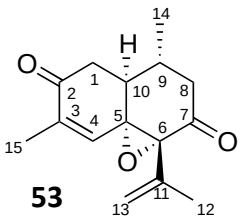
55

The spectroscopic data obtained was consistent with data reported in the literature.[180]

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.71 (m, 1H), 5.08 (m, 1H), 4.83 (s, 1H), 3.10 (br s, 1H, OH), 3.04 (s, *J* = 2.8 Hz, 1H), 2.63 (dd, *J* = 16.8, 7.2 Hz, 1H), 2.58 (dd, *J* = 14.7, 6.7 Hz, 1H), 2.54 (dd, *J* = 17.1, 5.1 Hz, 1H), 2.45 (dd, *J* = 14.6, 8.2 Hz, 1H), 2.33 (m, 1H), 2.14 (m, 1H), 1.85 (s, 3H), 1.82 (dd, *J* = 2.3, 1.4 Hz, 3H), 1.54 (s, 3H), 1.03 (d, *J* = 6.7 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 213.3, 198.6, 145.4, 142.8, 136.8, 115.8, 81.5, 46.3, 44.3, 41.4, 38.6, 32.4, 20.2, 19.9, 16.2.

(1a*R*,4*R*,4a*S*,8a*S*)-4,7-dimethyl-1a-(prop-1-en-2-yl)-3,4,4a,5-tetrahydro-1a*H*-naphtho[1,8a-*b*]oxirene-2,6-dione



53

HRMS Found M^+ 246.1254, $C_{15}H_{18}O_3$ requires M^+ 246.1260.

ν_{max} (NaCl): 2961, 2924, 2880, 1717 (C=O), 1684 (C=O), 1449, 1424, 1383, 997, 916 cm^{-1}.

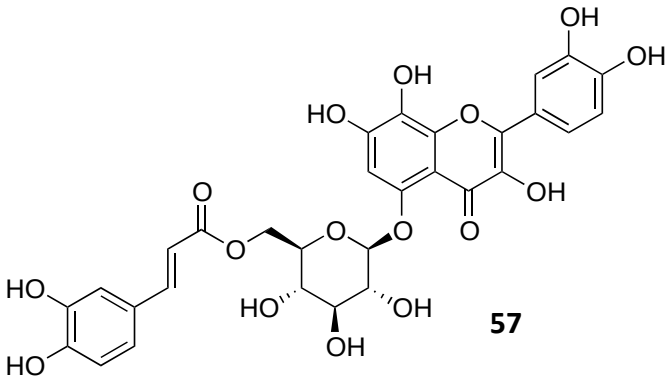
^{1}H NMR (600 MHz, $CDCl_3$): δ 6.01 (m, 1H, H^4), 5.26 (pent, *J* = 1.5 Hz, 1H, H^{13a}), 5.12 (m, 1H, H^{13b}), 2.90–2.83 (complex m, 2H, H^{1a} + H^{8a}), 2.46 (ddd, *J* = 13.6, 8.7, 5.1 Hz, 1H, H^{10}), 2.32 (dd, *J* = 15.8, 13.7 Hz, 1H, H^{1b}), 2.15 (dd, *J* = 13.2, 3.8

Hz, 1H, H^{8b}), 1.87–1.79 (complex m, 7H, H^{9} + H^{12} + H^{15}), 1.10 (d, *J* = 6.6 Hz, 3H, H^{14}).

^{13}C NMR (150 MHz, $CDCl_3$): δ	204.9, 197.2, 141.6, 141.5, 136.4, 116.9, 71.8, 70.3, 42.6, 42.5, 40.6, 39.0, 20.8, 20.5, 15.9.
MS (EI) m/z	246, 231, 191, 177, 163, 161, 149, 123, 107, 96, 79.
$[\alpha]_D^{22}$	+412 (c 0.05, $CHCl_3$)

(*E*)-((2*R*,3*S*,4*S*,5*R*,6*S*)-6-((2-(3,4-dihydroxyphenyl)-3,7,8-trihydroxy-4-oxo-4*H*-chromen-5-yl)oxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)methyl 3-(3,4-dihydroxyphenyl)acrylate

The spectroscopic data obtained was consistent with data reported in the literature.[185]

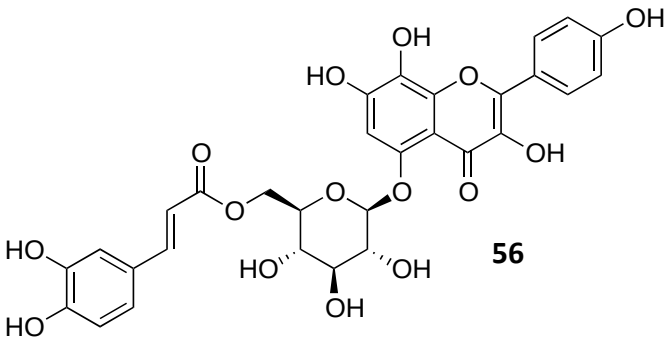


57

^{1}H NMR (600 MHz, MeOD): δ	7.71 (d, *J* = 2.2 Hz, 1H), 7.60 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.43 (d, *J* = 15.8 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 1H), 6.80 (s, 1H), 6.74 (d, *J* = 1.8 Hz, 1H), 6.56 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.53 (d, *J* = 8.2 Hz, 1H), 6.18 (d, *J* = 15.9 Hz, 1H), 5.08 (d, *J* = 7.6 Hz, 1H), 4.65 (dd, *J* = 12.0, 2.4 Hz, 1H), 4.33 (dd, *J* = 11.9, 7.1 Hz, 1H), 3.86 (m, 1H), 3.63 (t, *J* = 8.0 Hz, 1H), 3.58 (t, *J* = 9.2 Hz, 1H), 3.47 (t, *J* = 9.2 Hz, 1H).
^{13}C NMR (150 MHz, MeOD): δ	177.5, 169.1, 152.6, 150.3, 149.4, 148.93, 148.87, 147.6, 146.9, 146.5, 146.1, 136.2, 130.9, 127.4, 124.1, 122.2, 121.9, 116.5, 116.19, 116.18, 115.6, 114.5, 106.6, 102.1, 95.0, 77.4, 75.6, 74.5, 72.1, 64.6.

(*E*)-((2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5-trihydroxy-6-((3,7,8-trihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-5-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl 3-(3,4-dihydroxyphenyl)acrylate

The spectroscopic data obtained was consistent with data reported in the literature.[185]



56

^{1}H NMR (600 MHz, MeOD): δ 8.02 (d, *J* = 8.9 Hz, 2H), 7.43 (d, *J* = 15.8 Hz, 1H), 6.84–6.81 (complex m, 2H), 6.72 (d, *J* = 1.4 Hz, 1H), 6.58–6.50 (complex m, 2H), 6.15 (d, *J* = 15.9 Hz, 1H), 5.09 (d, *J* = 7.5 Hz, 1H), 4.69 (dd, *J* = 12.1, 2.3 Hz, 1H), 4.30 (dd, *J* = 12.1, 7.1 Hz, 1H), 3.87 (m, 1H), 3.63 (t, *J* = 8.2 Hz, 1H), 3.58 (t, *J* = 8.9 Hz, 1H), 3.46 (t, *J* = 9.3 Hz, 1H).

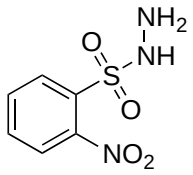
^{13}C NMR (150 MHz, MeOD): δ 177.5, 169.0, 160.6, 152.7, 150.3, 149.4, 149.0, 147.5, 146.9, 146.5, 137.1, 130.9, 130.8, 127.4, 123.6, 122.3, 116.4, 116.2, 115.4, 114.5, 106.7, 102.1, 95.0, 77.4, 75.6, 74.5, 72.1, 64.6.

Pyrethrin Extraction

Pyrethrin (*Chrysanthemum cinerariaefolium*) representative extraction

Dried, ground *C. cinerariaefolium* flowers (15.0 g) were placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200mL of a hot solution). This process was repeated a further two times, and the extracts combined before being extracted with heptane (3 x 200 mL). The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil. These extracts were used for reactions without further purification.

o-Nitrobenzenesulfonylhydrazide (NBSH)



70

To hydrazine hydrate (3.0 mL, 60 mmol, excess) at 0 °C was added *o*-nitrobenzenesulfonyl chloride **69** (2.0 g, 9.0 mmol) in THF (6 mL) dropwise. The reaction was warmed to rt and stirred for 30 min. Water (100 mL) was added, and the resulting suspension filtered to collect the precipitate. The resulting off–white crystals were washed with ice-cold water (2 x 20 mL) before being dried in a vacuum desiccator to provide the title compound as off–white crystals (1.56 g, 7.2 mmol, 80 %). The spectroscopic data obtained was consistent with data reported in the literature.[202]

^{1}H NMR (600 MHz, CD_3CN): δ 8.10 (m, 1H), 7.92–7.83 (complex m, 3H), 6.94 (br s, -NH, 1H), 4.02 (br s, -NH_2, 2H)

^{13}C NMR (150 MHz, CD_3CN): δ 149.2, 135.1, 133.1, 132.9, 130.6, 125.5.

Diimide test reactions

Eugenol

To *o*-nitrobenzenesulfonylhydrazide (217 mg, 1.0 mmol) and K_3PO_4 (106 mg, 0.5 mmol) in CH_3CN (4 mL) was added eugenol (164 mg, 1.0 mmol) dropwise. The reaction was stirred at rt for 20 h before water (50 mL) was added and the reaction extracted with heptane (3 x 15 mL). The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil (169 mg).

Anethole

To *o*-nitrobenzenesulfonylhydrazide (217 mg, 1.0 mmol) and K_3PO_4 (106 mg, 0.5 mmol) in CH_3CN (4 mL) was added anethole (148 mg, 1.0 mmol) dropwise. The reaction was stirred at rt for 20 h before water (50 mL) was added and the reaction extracted with heptane (3 x 15 mL). The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil (107 mg).

Pyrethrin diimide reactions

Test conditions I

C. cinerariaefolium flowers (3 x 15.0 g) were extracted by the general method above to yield a pale yellow/green oil (590 mg). To *o*-nitrobenzenesulfonylhydrazide (659 mg, 3.04 mmol) and K_3PO_4 (322 mg, 1.52 mmol) in CH_3CN (8 mL) was added the extract in CH_3CN (5 mL) dropwise. The reaction was stirred at rt for 21 h before water (50 mL) was added and the reaction extracted with heptane (3 x 15 mL). The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil (500 mg).

Test conditions II

C. cinerariaefolium flowers (3 x 15.0 g) were extracted by the general method above to yield a pale yellow/green oil (722 mg). To *o*-nitrobenzenesulfonylhydrazide (250 mg, 1.15 mmol) and K_3PO_4 (250 mg, 1.18 mmol) in CH_3CN (5 mL) was added the extract in CH_3CN (10 mL) dropwise. The reaction was stirred at rt for 18 h before 0.2 mL was taken, diluted with water (1 mL) and extracted with heptane (2 mL). The solvent was evaporated and the sample analysed by NMR and GC-MS. To the rest of the reaction was added *o*-nitrobenzenesulfonylhydrazide (150 mg, 0.69 mmol) and K_3PO_4 (150 mg, 0.71 mmol) and stirred for a further 48 h. Water (100 mL) was added and the reaction extracted with heptane (5 x 20 mL). The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil (546 mg).

Optimised conditions

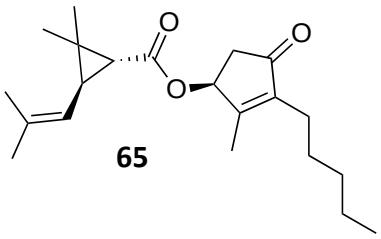
C. cinerariaefolium flowers (3 x 15.0 g) were extracted by the general method above to yield a pale yellow/green oil (721 mg). To *o*-nitrobenzenesulfonylhydrazide (250 mg, 1.15 mmol) and K_3PO_4 (250 mg, 1.18 mmol) in CH_3CN (5 mL) was added the extract in CH_3CN (10 mL) dropwise. The reaction was stirred at rt for 42 h. Water (100 mL) was added and the reaction extracted with heptane (5 x 20 mL). The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil (564 mg).

Synthesis of Dihydro Derivatives

C. cinerariaefolium flowers (3 x 15.0 g) were extracted by the general method above to yield a pale yellow/green oil (700 mg). To *o*-nitrobenzenesulfonylhydrazide (800 mg, 3.68 mmol) and K_3PO_4 (600 mg, 2.83 mmol) in CH_3CN (10 mL) was added the extract in CH_3CN (10 mL) dropwise. The reaction was stirred at rt for 72 h. Water (100 mL) was added and the reaction extracted with heptane (5 x 20 mL). The combined organic fractions were dried over $MgSO_4$, filtered and evaporated to dryness to yield a crude pale green oil (429 mg). The ensuing mixture was fractionated via automated gradient flash chromatography (silica; 0 → 40 % EtOAc/hexane) to yield mixtures of dihydrojasmolin I dihydrocinerin I; and dihydrojasmolin II and dihydrocinerin II. These fractions were analysed by HRMS.

Dihydro derivative HRMS

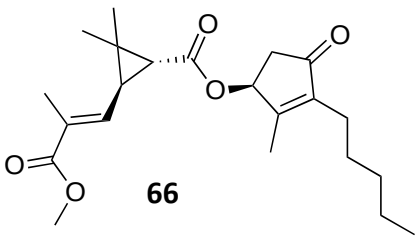
Dihydrojasmolin I



65

HRMS Found $[M+Na]^+$ 355.2241, $C_{21}H_{32}O_3Na^+$ requires M^+ 355.2244.

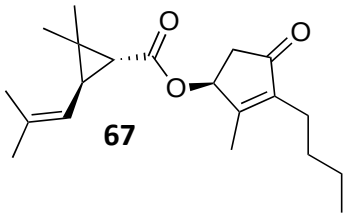
Dihydrojasmolin II



66

HRMS Found $[M+Na]^+$ 399.2135, $C_{22}H_{32}O_5Na^+$ requires M^+ 399.2142.

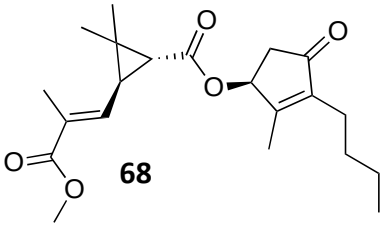
Dihydrocinerin I



67

HRMS Found $[M+Na]^+$ 341.2084, $C_{20}H_{30}O_3Na^+$ requires M^+ 341.2087.

Dihydrocinerin II



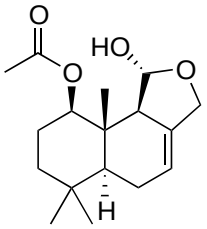
68

HRMS Found $[M+Na]^+$ 385.1980, $C_{21}H_{30}O_5Na^+$ requires M^+ 385.1985.

1β-acetoxypolygodial survey (Wielangta forest *T. lanceolata*) representative extraction

Dried, ground *T. lanceolata* leaves (15 g) were mixed with sand (4 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). This process was completed 1–3 times depending on the mass of each specimen. The extract was concentrated to a ~half volume under reduced pressure in order to remove EtOH (50 °C water bath temperature). The ensuing mixture was extracted with heptane (4 × 50 or 4 x 100 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a viscous, green oil. Purification via automated gradient flash column chromatography (silica; 0 → 100% EtOAc/hexane) provided the three compounds **12**, **80** and **81**.

1β-Acetoxyisodrimeninol



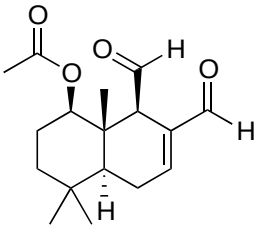
81

^{1}H NMR (600 MHz, $CDCl_3$): δ — 5.52 (s, 1H), 5.44 (m, 1H), 4.62 (dd, *J* = 11.7, 4.3 Hz, 1H), 4.40 (d, *J* = 11.1 Hz, 1H), 4.12 (m, 1H), 2.27 (m, 1H), 2.16 (m, 1H), 2.05 (s, 3H), 2.01 (m, 1H), 1.73 (dq, *J* = 12.9, 4.0 Hz, 1H), 1.64 (m, 1H), 1.52–1.41 (complex m, 2H), 1.37 (dd, *J* = 11.6, 5.4 Hz, 1H), 0.94 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ — 171.0, 136.5, 116.7, 99.7, 81.3, 68.3, 60.7, 49.1, 39.8, 37.7, 32.6, 32.5, 24.3, 23.3, 21.5, 21.4, 9.2.

$[\alpha]_D^{20}$ — –28 (c 0.35, $CHCl_3$), lit. $[\alpha]_D$ +10 (c 0.06, $CHCl_3$)[343*]

1β-Acetoxypolygodial



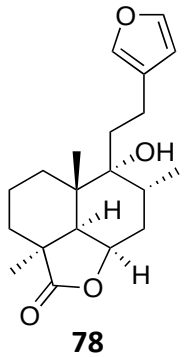
80

^{1}H NMR (600 MHz, $CDCl_3$): δ — 9.74 (d, *J* = 3.6 Hz, 1H), 9.37 (s, 1H), 7.08 (dt, *J* = 5.6, 2.3 Hz, 1H), 4.74 (dd, *J* = 11.6, 4.2 Hz, 1H), 3.17 (m, 1H), 2.49 (m, 1H), 2.40 (dddd, *J* = 20.0, 11.8, 3.6, 2.5 Hz, 1H), 2.05 (s, 3H), 1.80 (dq, *J* = 13.1, 3.7 Hz, 1H), 1.63 (m, 1H), 1.55 (dt, *J* = 13.7, 3.5 Hz, 1H), 1.48 (td, *J* = 13.7, 3.5 Hz, 1H), 1.38 (dd, *J* =11.8, 4.7 Hz, 1H), 1.04 (s, 3H), 1.00 (s, 3H), 0.95 (s, 3H).

* Incorrectly assigned, corrected in this work. See Deans *et. al* 2014[105]

^{13}C NMR (150 MHz, $CDCl_3$): δ	200.1, 192.5, 170.3, 152.6, 139.5, 81.1, 59.5, 48.7, 41.9, 39.1, 32.8, 32.6, 24.7, 24.1, 22.1, 21.4, 10.5.
$[\alpha]_D^{20}$	+16 (c 0.1, $CHCl_3$).

Marrubiin from White Horehound (*Marrubium vulgare*)



78

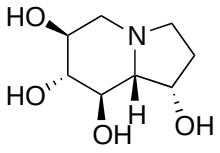
Dried, ground *M. vulgare* (15.0 g) was placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH:H_2O (200 mL of a hot solution). This process was repeated on a further 3 x 15.0 g of material. The ensuing mixture was extracted with heptane (3 x 200 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a viscous, green oil (862 mg). Purification via automated gradient flash column chromatography (silica; 0 → 60 % EtOAc/hexane) provided the title compound as a white amorphous solid (8 mg, 0.05 % w/w) with a further 228 mg of impure material. The spectroscopic data obtained was consistent with data reported in the literature.[226]

^{1}H NMR (600 MHz, $CDCl_3$): δ	7.36 (m, 1H), 7.24 (s, 1H), 6.27 (s, 1H), 4.74 (m, 1H), 2.53 (m, 2H), 2.23 (d, *J* = 4.7 Hz, 1H), 2.20–2.07 (complex m, 3H), 1.90 (m, 1H), 1.80–1.64 (complex m, 4H), 1.56–1.41 (complex m, 2H), 1.36–1.25 (complex m, 4H), 1.06 (s, 3H), 0.97 (d, *J* = 6.5 Hz, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	183.8, 143.1, 138.6, 125.1, 110.7, 76.2, 75.8, 44.9, 43.8, 39.7, 35.1, 32.4, 31.5, 28.6, 28.4, 23.0, 22.3, 21.0, 18.2, 16.6.

Crude Extract from Bitter Melon (Momordia charantia)

Fresh whole *M. charantia* was sliced and dried in an oven at 35 °C. The slices were ground to a coarse powder and 4 g of this preparation was mixed with 12 g of acid washed sand, placed into the portafilter (sample compartment) of an espresso machine and extracted with H_2O (200 mL). The extract was evaporated to dryness under reduced pressure at 50 °C. Typical yields were 1 g extract per 4 g (0.25 % w/w).

Castanospermine from Moreton Bay Chestnuts (*Castanospermum australe*)



79

Fresh *C. australe* seeds (20 g) were grated with a cheese grater and mixed with sand (10 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 30% EtOH/H_2O (200 mL of a hot solution). This process was completed on a further 4 samples. The ensuing mixture was concentrated to half volume under reduced pressure (bath temperature 60 °C) before being filtered through a sintered glass funnel. Amberlyst® 15 acidic ion exchange resin (30 g) was added and the mixture stirred for 45 min before being filtered and the resin washed with water. The resin was then eluted with 15% NH_3:MeOH (5 x 60 mL), the solvents were removed under reduced pressure to yield a crude light orange oil (2.15 g). Purification via automated gradient flash chromatography (silica, 0 → 60% (5% NH_3:MeOH):CH_2Cl_2) provided the title compound as a white amorphous solid (565 mg, 0.57% yield w/w). The spectroscopic data obtained was consistent with data reported in the literature.[319]

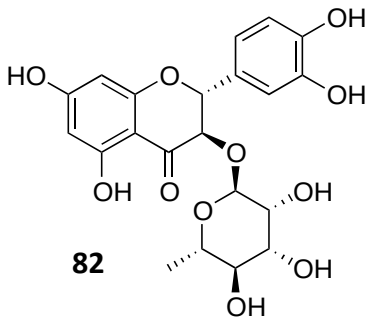
^{1}H NMR (400 MHz, D_2O): δ	4.34 (m, 1H), 3.59–3.49 (complex m, 2H), 3.26 (t, *J* = 9.2 Hz, 1H), 3.11 (dd, *J* = 10.9, 5.1 Hz, 1H), 3.02 (td, *J* = 8.9, 2.0 Hz, 1H), 2.27 (m, 1H), 2.15 (q, *J* = 9.1 Hz, 1H), 2.04–1.94 (complex m, 2H), 1.64 (m, 1H).
^{13}C NMR (100 MHz, D_2O): δ	78.9, 71.3, 70.0, 69.5, 68.9, 55.3, 51.5, 32.7.

Glycosides from *Drimys winteri*

Dried, ground *D. winteri* leaves (17.1 g) were placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (250 mL of a hot solution). The ensuing mixture was extracted with heptane (4 × 100 mL), followed by EtOAc (3 x 100 mL). The EtOAc fractions were then combined, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to yield a crude yellow/brown solid (2.07 g). This material was then partially purified by normal phase automated gradient flash chromatography (silica; 0 → 20 % MeOH/CH_2Cl_2) to yield a combined fraction of astilbin and quercitrin as a fine yellow powder (1.44 g) containing astilbin (1.0 g, 5.8 % w/w) and quercitrin (0.44 g, 2.6 % w/w). Purification of a small portion via reversed-phase automated gradient flash column chromatography (C_{18} silica; 0 → 40% CH_3CN:H_2O) yielded the products as white amorphous solids for characterisation.

Astilbin

The spectroscopic data obtained was consistent with data reported in the literature.[242]



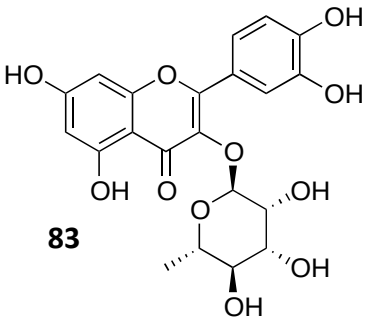
82

^{1}H NMR (600 MHz, MeOD): δ 6.98 (d, *J* = 1.8 Hz, 1H), 6.87–6.82 (complex m, 2H), 5.94 (d, *J* = 2.1 Hz, 1H), 5.92 (d, *J* = 2.1 Hz, 1H), 5.09 (d, *J* = 10.7 Hz, 1H), 4.59 (d, *J* = 10.7 Hz, 1H), 4.27 (m, 1H), 4.08 (m, 1H), 3.69 (dd, *J* = 9.6, 3.3 Hz, 1H), 3.57 (dd, *J* = 3.1, 1.6 Hz, 1H), 3.33 (m, 1H), 1.21 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR (150 MHz, MeOD): δ 194.6, 167.2, 164.1, 162.7, 146.0, 145.1, 127.8, 119.1, 115.0, 114.1, 101.1, 100.7, 96.0, 94.9, 82.5, 77.2, 72.4, 70.8, 70.4, 69.1, 16.5.

Quercitrin

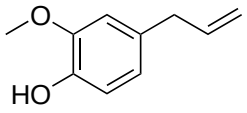
The spectroscopic data obtained was consistent with data reported in the literature.[243]



83

^{1}H NMR (600 MHz, MeOD) δ	7.36 (d, *J* = 2.1 Hz, 1H), 7.33 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 6.39 (d, *J* = 2.0 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 5.37 (d, *J* = 1.3 Hz, 1H), 4.24 (d, *J* = 3.2, 1.7 Hz, 1H), 3.77 (dd, *J* = 9.4, 3.3 Hz, 1H), 3.44 (dq, *J* = 9.6, 6.1 Hz, 1H), 3.36 (d, *J* = 9.6 Hz, 1H), 0.96 (d, *J* = 6.1 Hz, 3H).
^{13}C NMR (150 MHz, MeOD) δ	178.3, 164.6, 161.8, 157.9, 157.2, 148.1, 145.0, 134.8, 121.6, 121.5, 115.5, 115.0, 104.5, 102.2, 98.4, 93.3, 71.9, 70.7, 70.6, 70.5, 16.2.

Eugenol from cloves (*Syzygium aromaticum*)



73

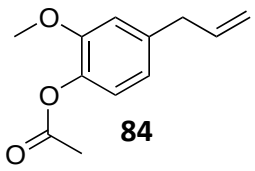
Ground *S. aromaticum* flower buds (cloves) (15 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 25% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with heptane (3 x 50 mL). The organic fractions were then combined and extracted with NaOH (2 M aq., 3 x 30 mL). The combined aqueous extracts were acidified with HCl (conc.) until cloudy, before being split into two portions. These portions were extracted with CH_2Cl_2 and heptane respectively (3 x 20 mL) before each was dried ($MgSO_4$), filtered, and the solvent

removed under reduced pressure to provide a pale yellow oil (646 mg and 636 mg respectively, total combined yield of 8.5 % w/w). The spectroscopic data obtained was consistent with data reported in the literature.[320]

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.85 (m, 1H), 6.69 (m, 2H), 5.96 (m, 1H), 5.50 (s, 1h, OH), 5.07 (m, 2H), 3.88 (s, 2H), 3.33 (d, *J* = 6.8 Hz, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 146.6, 144.1, 138.0, 132.1, 121.4, 115.7, 114.4, 111.3, 56.0, 40.1.

Acetyleugenol from cloves (*Syzygium aromaticum*)



84

Ground *S. aromaticum* flower buds (cloves) (15 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 25% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with heptane (3 x 50 mL). The organic fractions were then combined and extracted with NaOH (2 M aq., 3 x 30 mL) which was then discarded. The remaining heptane fraction was dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a pale-yellow oil (181 mg, 1.2 % w/w). The spectroscopic data obtained was consistent with data reported in the literature.[321,322]

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97 (d, *J* = 8.0 Hz, 1H), 6.80 (m, 2H), 5.99 (m, 1H), 5.18–5.09 (complex m, 2H), 3.82 (s, 3H), 3.38 (d, *J* = 6.7 Hz, 2H), 2.31 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 150.9, 139.0, 138.0, 137.0, 122.5, 120.7, 116.2, 112.7, 55.8, 40.1, 20.7.

Crude extract from Star anise (*Illicium verum*)

Dried, ground *I. verum* seed pods (15.0 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 25% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with CH_2Cl_2 (3 x 50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a yellow/green oil (574 mg).

Crude extract from Caraway seeds (*Carum carvi*)

Dried, ground *C. carvi* seeds (15.0 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 30% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with CH_2Cl_2 (3 x 50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a yellow oil (127 mg).

Crude extract from Oregano (*Origanum vulgare*) I

Dried, ground *O. vulgare* leaves (15.0 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 30% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with CH_2Cl_2 (3 x 50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a yellow/orange oil (204 mg).

Crude extract from Oregano (*Origanum vulgare*) II

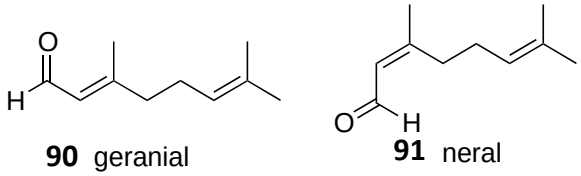
Dried, ground *O. vulgare* leaves (12.5 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 25% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with CH_2Cl_2 (3 x 50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a yellow/orange oil (61 mg).

Crude extract from Thyme (*Thymus vulgaris*)

Dried, ground *T. vulgaris* leaves (15.0 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 30% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with CH_2Cl_2 (3 x

50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a yellow oil (219 mg).

Citral from Lemon Myrtle (*Backhousia citriodora*)



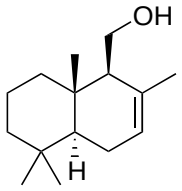
90 geranial **91** neral

Dried, ground *B. citriodora* leaves (15 g) were mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted with 35% EtOH/H_2O (200 mL of a hot solution). The ensuing mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic fractions were then combined, dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide a pale yellow oil (1.21 g). Purification via automated gradient flash chromatography (silica; 0 → 60% EtOAc/hexane) provided the title mixture of compounds (~2:1 **90**:**91**) as pale yellow oil (630 mg, 4.2% yield w/w). The spectroscopic data obtained was consistent with data reported in the literature.[323,324]

5.4 Chapter 4 Experimental

Polygodial derivatives

(−)-Drimenol



118

5% Pd/C (15 mg) was added to a solution of drimendiol (**15**) (180 mg, 0.76 mmol) in EtOH (25 mL). The resulting suspension was maintained under H_2 (1 atm) for 1.25 h then filtered through Celite™ and the solvent was removed under reduced pressure. Purification via automated flash gradient column chromatography (silica; 0 → 25% EtOAc/hexane) provided the title compound as a colourless solid (107 mg, 64% yield). The spectroscopic data obtained was consistent with data reported in the literature.[278]

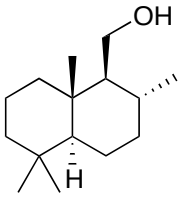
^{1}H NMR (400 MHz, $CDCl_3$): δ	5.54 (m, 1H), 3.85 (dd, *J* = 11.3, 3.4 Hz, 1H), 3.73 (dd, *J* = 11.3, 4.9 Hz, 1H), 2.01–1.75 (complex m, 8H), 1.64–1.38 (complex m, 4H), 1.29 (br s, 1H), 1.22–1.12 (complex m, 1H), 1.06 (td, *J* = 13.2, 3.8 Hz, 1H), 0.89 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	133.1, 124.1, 61.0, 57.3, 42.2, 39.9, 36.1, 33.4, 33.0, 23.7, 22.1, 22.0, 18.9, 15.0.
$[\alpha]_D^{22}$	−13 (c 1.0, $CHCl_3$), lit. $[\alpha]_D$ −15 (c 1.0, $CHCl_3$)[278]

((1*S*,2*R*,4a*S*,8a*S*)-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)methanol,
((1*S*,2*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)methanol

A solution of drimendiol (**15**) (1.5 g, 6.29 mmol) in EtOH (40 mL) was subjected to a hydrogen atmosphere (1 atm) under a balloon with catalytic Pd/C for 5 h. The resulting mixture was filtered through Celite™ and the solvent evaporated to yield a pale-yellow oil (1.16 g). Purification via automated gradient flash column chromatography (silica; 0 → 10 % EtOAc/hexane) yielded the title compounds as white solids (**119** 251 mg, 1.12 mmol, 18 % yield and **120** 164 mg, 0.73 mmol, 12 % yield)

((1*S*,2*R*,4a*S*,8a*S*)-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)methanol

The spectroscopic data obtained was consistent with data reported in the literature.[281]

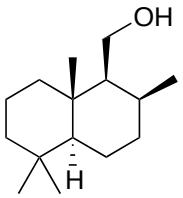


119

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.78 (dd, *J* = 11.6, 3.2 Hz, 1H), 3.62 (dd, *J* = 11.6, 3.5 Hz, 1H), 1.88 (m, 1H), 1.79 (dq, *J* = 13.0, 3.2 Hz, 1H), 1.67–1.52 (complex m, 3H), 1.50–1.36 (complex m, 2H), 1.30 (qd, *J* = 12.8, 3.7 Hz, 1H), 1.15 (td, *J* = 13.4, 3.8 Hz, 1H), 1.08–0.95 (complex m, 4H), 0.87 (s, 3H), 0.86–0.81 (complex m, 7H), 0.68 (dt, *J* = 11.2, 3.2 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 61.8, 60.6, 55.0, 42.1, 39.4, 37.6, 36.8, 33.6, 33.3, 30.8, 21.9, 21.8, 21.0, 18.8, 15.6.

((1*S*,2*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)methanol



120

The spectroscopic data obtained was consistent with data reported in the literature.[280,281]

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (dd, *J* = 10.7, 4.5 Hz, 1H), 3.58 (dd, *J* = 10.6, 9.5 Hz, 1H). 2.16 (m, 1H), 1.73–1.33 (complex m, 9H), 1.17 (td, *J* = 13.9, 4.6 Hz, 1H), 1.02 (td, *J* = 13.0, 3.7, 1H), 0.97 (d, *J* = 7.5 Hz, 3H), 0.90–0.85 (complex m, 7H), 0.82 (s, 3H).

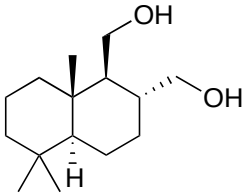
^{13}C NMR (100 MHz, $CDCl_3$): δ 60.9, 56.5, 55.7, 42.0, 39.9, 37.6, 34.5, 33.6, 33.2, 28.5, 21.6, 18.4, 17.5, 17.0, 15.6.

((1*S*,2*R*,4a*S*,8a*S*)-5,5,8a-trimethyldecahydronaphthalene-1,2-diyl)dimethanol, ((1*S*,2*S*,4a*S*,8a*S*)-5,5,8a-trimethyldecahydronaphthalene-1,2-diyl)dimethanol

To a solution of polygodial (**12**) (1.7 g, 7.25 mmol) in EtOH (40 mL) at 0 °C was added slowly $NaBH_4$ (1.2 g, 31.7 mmol). The resulting mixture was stirred at 0 °C for 30 min and rt for 1 h. The solvent was evaporated and the residue taken up in water (50 mL) and extracted with CH_2Cl_2 (4 x 20 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield a pale-yellow oil containing drimendiol (**15**) (1.75 g) used without further purification.

Crude drimendiol (**15**) (525 mg) was dissolved in EtOH and subjected to a hydrogen atmosphere (1 atm) under a balloon with catalytic Pd/C for 8 h. The resulting mixture was filtered through Celite™ and the solvent evaporated to yield a pale-yellow oil (490 mg). Purification via automated gradient flash column chromatography (silica; 0 → 100 % EtOAc/hexane) provided the title compounds as white solids (**122**, 80 mg, 0.33 mmol, 15 % over 2 steps and **123**, 220 mg, 0.92 mmol, 41 % yield over 2 steps)

((1*S*,2*R*,4a*S*,8a*S*)-5,5,8a-trimethyldecahydronaphthalene-1,2-diyl)dimethanol



122

HRMS Found $[M+Na]^+$ 263.1987, $C_{15}H_{28}O_2Na$ requires M^+ 263.1982.

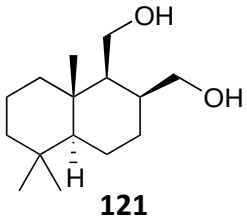
ν_{max} (NaCl): 3233 (br, -OH), 2993, 2931, 2841, 1462, 1437, 1384, 1367, 1056, 1021, 981, 949, 758 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.95 (dd, *J* = 11.0, 1.6 Hz, 1H), 3.78 (dd, *J* = 11.4, 2.8 Hz, 1H), 3.58 (dd, *J* = 11.0, 6.9 Hz, 1H), 3.51 (dd, *J* = 11.4, 5.1 Hz, 1H), 3.11 (br m, 2H, OH), 1.92 (m, 1H),

	1.77–1.01 (complex m, 11H), 0.90–0.84 (complex m, 7 H), 0.83 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	67.5, 62.4, 56.4, 54.7, 42.0, 40.0, 39.1, 37.2, 33.5, 33,4, 31.0, 21.7, 21.6, 18.7, 15.3.
$[\alpha]_D^{20}$	+26 (c 1.35, $CHCl_3$), lit. $[\alpha]_D$ +26 (c 3.1, $CHCl_3$)[325]

((1*S*,2*S*,4a*S*,8a*S*)-5,5,8a-trimethyldecahydronaphthalene-1,2-diyl)dimethanol

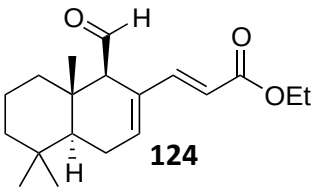
The spectroscopic data obtained was consistent with data reported in the literature.[282]



121

^{1}H NMR (400 MHz, $CDCl_3$): δ	3.85 (dd, *J* = 10.4, 6.2 Hz, 1H), 3.75 (dd, *J* = 11.1, 5.1 Hz, 1H), 3.67 (dd, *J* = 11.1, 10.2 Hz, 1H), 3.52 (dd, *J* = 10.4, 7.1 Hz, 1H), 2.11 (quint, *J* = 5.8 Hz, 1H), 1.85 (m, 1H), 1.56 (m, 1H), 1.53–1.39 (complex m, 4H), 1.38–1.28 (complex m, 2H), 1.18 (m, 1H), 1.10 (td, *J* = 13.5, 4.0 Hz, 1H), 0.95 (td, *J* = 9.1, 3.8 Hz, 1H), 0.83 (dd, *J* = 12.1, 2.0 Hz, 1H), 0.80 (s, 3H), 0.79 (s, 3H), 0.74 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	64.2, 60.8, 56.4, 54.4, 41.9, 39.4, 37.9, 37.4, 33.5, 33.2, 30.1, 21.6, 18.44, 18.42, 16.5.

(*E*)-Ethyl 3-((1*R*,4a*S*,8a*S*)-1-formyl-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl)acrylate

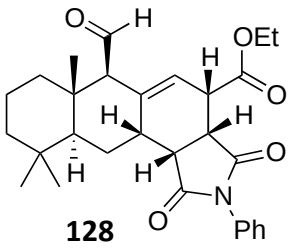


124

Ethyl (triphenylphosphoranylidene)acetate (**123**) (208 mg, 0.60 mmol) was added to a solution of polygodial (**12**) (101 mg, 0.43 mmol) in EtOH (15 mL). The reaction was heated to 40 °C and after 2.5 h, the mixture was cooled, and the solvent was removed under reduced pressure. The ensuing residue was purified via flash chromatography (silica; 0 → 15% EtOAc/hexane) to provide the title compound as a colourless oil (102 mg, 79% yield).

HRMS	Found M^+ 304.2030, $C_{19}H_{28}O_3$ requires M^+ 304.2038.
ν_{max} (NaCl):	2928, 1713, 1627, 1368, 1311, 1712, 1040 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ	9.46 (d, *J* = 4.8 Hz, 1H), 7.30 (d, *J* = 16.3 Hz, 1H), 6.49 (m, 1H), 5.48 (d, *J* = 16.3 Hz, 1H), 4.15 (q, 7.2 Hz, 2H), 2.81 (s, 1H), 2.31 (m, 1H), 2.20 (m, 1H), 1.83 (m, 1H), 1.56–1.42 (complex m, 3 H), 1.38–1.13 (complex m, 6H), 0.99 (s, 3H), 0.93 (s, 3H), 0.89 (2, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	205.4, 167.1, 146.4, 141.5, 130.5, 116.7, 62.9, 60.5, 48.8, 41.8, 40.3, 37.5, 33.3, 33.2, 24.8, 22.3, 18.1, 15.5, 14.2.
MS (EI) m/z	304 (M^+), 275, 259, 231, 229, 215, 103, 180, 166, 145, 123, 109, 95, 91, 81, 69, 55.
$[\alpha]_D^{22}$	−8.4 (c 1.1, $CHCl_3$).

(3a*R*,4*S*,6*R*,6a*S*,10a*S*,11a*S*,11b*R*)-Ethyl 6-formyl-6a,10,10- trimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,6a,7,8,9,10,10a,11,11a,11b-tetradecahydro-1H-naphtho[2,3-*e*]isoindole-4-carboxylate



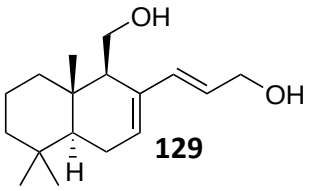
128

N-Phenylmaleimide (**127**) (13.7 mg, 0.079 mmol) was added to a solution of Wittig product **124** (22 mg, 0.072 mmol) in toluene (10 mL) and the resulting solution was then heated at reflux. After 18 h, the reaction mixture was cooled, and the solvent was removed under reduced pressure. The ensuing residue was then purified by flash column chromatography (silica; 30% EtOAc/hexane) to provide the title compound as a light-yellow oil (19 mg, 55% yield).

HRMS	Found M^+ 477.2508, $C_{29}H_{35}NO_5$ requires M^+ 477.2515.
ν_{max} (NaCl):	2928, 1737, 1712, 1500, 1386, 1207, 1195, 754 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ	9.58 (d, 4.7 Hz, 1H), 7.43 (m, 2H), 7.36 (m, 1H), 7.10 (m, 2H), 5.98 (m, 1H), 4.29 (m, 2H), 3.84 (dd, *J* = 8.8, 6.0 Hz, 1H), 3.34 (dd, *J* = 8.8, 6.0 Hz, 1H), 3.27 (m, 1H), 2.74 (m, 1H), 2.52 (ddd, *J* = 14.4, 4.8, 1.3 Hz, 1H), 2.47 (m, 1H), 1.99 (m, 1H), 1.57–1.39 (complex m, 4H), 1.31 (t, *J* = 7.1 Hz, 3H), 1.30–1.19 (m, 3H), 1.10 (s, 3H), 0.98 (s, 3H), 0.92 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	202.5, 176.6, 175.7, 170.2, 137.1, 131.6, 129.4, 129.0, 126.7, 123.5, 67.3, 61.6, 48.5, 45.7, 43.6, 42.1, 41.8, 40.1, 36.8, 33.7, 33.6, 32.7, 22.4, 21.9, 18.4, 17.3, 14.3.

MS (EI) m/z	477 (M^+), 462, 459, 432, 403, 402, 374, 373, 304, 174, 137, 123, 109, 91, 81, 79, 69, 55.
$[\alpha]_D^{22}$	+73 (c 0.9, $CHCl_3$).

(*E*)-3-((1*R*,4a*S*,8a*S*)-1-(hydroxymethyl)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl)prop-2-en-1-ol

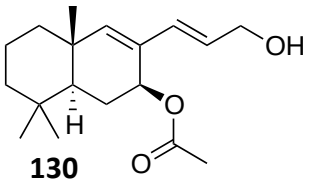


129

To a solution of the Wittig product **124** (130 mg, 0.43 mmol) in dry THF under N_2 at 0 °C was added $LiAlH_4$ (130 mg, 3.43 mmol). The reaction was warmed to rt and stirred for 1 h. The reaction was quenched at 0 °C with water (130 µL), KOH (130 µL, 3 M) and water (400 µL). The reaction mixture was then dried ($MgSO_4$), filtered and evaporated to yield a colourless oil (92 mg). Purification via automated gradient flash column chromatography (silica; 0 → 80 % EtOAc/hexane) provided the title compound as an impure colourless oil (28 mg).

^{1}H NMR (600 MHz, $CDCl_3$): δ	6.35 (d, 15.6 Hz, 1H), 5.93 (m, 1H), 5.90 (dt, *J* = 15.6, 5.9 Hz, 1H), 4.16 (d, *J* = 5.8 Hz, 2H), 3.93 (dd, *J* = 11.7, 3.8 Hz, 1H), 3.73 (dd, *J* = 11.7, 4.6 Hz, 1H), 2.13 (m, 1H), 2.07 (s, 1H), 2.03–1.95 (complex m, 2H), 1.59 (qt, *J* = 13.7, 3.1 Hz, 1H), 1.52–1.42 (complex m, 2H), 1.24–1.16 (complex m, 2H), 1.09 (dd, *J* = 13.3, 3.4 Hz, 1H), 0.92 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	135.5, 133.2, 127.3, 127.2, 63.7, 60.3, 55.4, 49.5, 42.1, 39.9, 36.0, 33.3, 32.9, 23.9, 22.1, 18.7, 14.7.

(2*S*,4a*R*,8a*S*)-3-((*E*)-3-hydroxyprop-1-en-1-yl)-4a,8,8-trimethyl-1,2,4a,5,6,7,8,8a-octahydronaphthalen-2-yl acetate



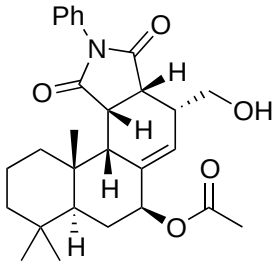
130

To a solution of diol **129** (28 mg, 0.11 mmol) in CH_2Cl_2 (10 mL) was added $Pb(OAc)_4$ (50 mg, 0.11 mmol). The reaction was stirred for 16 h before being filtered through a short silica plug eluting with EtOAc. Evaporation of the solvent yielded the crude product as a colourless oil (30 mg). Purification via automated gradient flash column chromatography (silica; 0 → 30 % EtOAc/hexane) provided the title compound as an impure colourless oil (16 mg). Used without full characterisation. Reaction product consistent with published reactions[283] and NMR studies.[326]

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.07 (d, *J* = 16.4 Hz, 1H), 5.73 (s, 1H), 5.72–5.61 (complex m, 2H), 4.16 (d, *J* = 5.9 Hz, 2H), 2.35 (dd, *J* = 12.9, 8.0, 2.0 Hz, 1H), 2.09 (s, 3H), 1.67 (m, 1H), 1.56–1.43 (complex m, 4H), 1.26–1.17 (complex m, 3H), 1.09 (s, 3H), 0.91 (s, 3H), 0.86 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 171.0, 147.4, 131.0, 130.3, 127.0, 71.2, 63.9, 49.2, 41.7, 39.2, 35.9, 32.8, 32.7, 26.1, 21.4, 21.1, 20.3, 18.7.

(3a*R*,4*R*,6*S*,7a*S*,11a*S*,11b*S*,11c*S*)-4-(hydroxymethyl)-8,8,11a-trimethyl-1,3-dioxo-2-phenyl-2,3,3a,4,6,7,7a,8,9,10,11,11a,11b,11c-tetradecahydro-1*H*-naphtho[1,2-*e*]isoindol-6-yl acetate



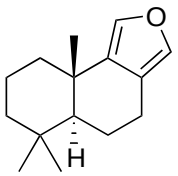
131

To a solution of hydroxy acetate **130** (16 mg, 0.054 mmol) in toluene (10 mL) was added *N*-phenylmaleimide (**127**) (20 mg, 0.12 mmol). The reaction was stirred under reflux for 16 h before the solvent was evaporated. Purification via automated gradient flash column chromatography (silica; 0 → 75 % EtOAc/hexane) provided the title compound as a colourless oil (14 mg, 0.030 mmol, 7% yield over 3 steps).

HRMS	Found M^+ 465.2509, $C_{28}H_{35}O_5N$ requires M^+ 465.2515.
ν_{max} (NaCl):	3460 (br, -OH), 2953, 2924, 2868, 1733, 1699 (C=O), 1500, 1383, 1237, 1200, 1033, 752 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	7.48 (m, 2H), 7.41 (tt, *J* = 7.5, 1.2 Hz, 1H), 7.17 (m, 2H), 5.59 (dt, *J* = 4.1, 2.4 Hz, 1H), 5.4 (m, 1H), 4.07 (m, 1H), 3.93 (ddd, *J* = 12.0, 10.0, 5.7 Hz, 1H), 3.72 (dd, *J* = 8.5, 3.9 Hz, 1H), 3.51 (t, *J* = 8.2 Hz, 1H), 3.04 (dd, *J* = 8.6, 5.9 Hz, 1H), 2.74 (m, 1H), 2.19 (m, 1H), 2.17–2.10 (complex m, 5H), 1.93 (ddd, *J* = 12.4, 7.0, 1.5 Hz, 1H), 1.71–1.40 (complex m, 6H), 1.18 (s, 3H), 0.96 (s, 3H), 0.90 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	177.8, 177.3, 170.6, 140.4, 131.6, 129.4, 129.0, 126.9, 124.0, 72.4, 62.6, 51.8, 43.6, 43.5, 43.4, 41.1, 39.4, 37.5, 35.6, 33.6, 33.3, 26.42, 26.35, 22.5, 21.3, 18.9.

MS (EI) m/z	465 (M^+), 405, 375, 360, 306, 278, 232, 218, 175, 161, 119, 109, 95, 93.
$[\alpha]_D^{20}$	–10 (c 0.7, $CHCl_3$).

(+)-Euryfuran



133

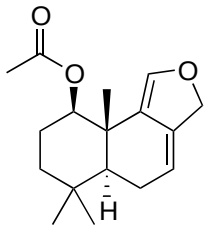
$NaBH_4$ (27 mg, 0.71 mmol) was added to a solution of polygodial (**12**) (83 mg, 0.35 mmol) in EtOH (20 mL) at 0 °C. After 0.33 h H_2O (30 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (3 × 30 mL) to provide a pale-yellow oil (74 mg). This residue was subjected to flash chromatography (silica; 0 → 30% EtOAc/hexane over 10 min) to afford lactol **132** (21 mg) which still contained impurities. HCl (2 M, 2 drops) was then added to a solution of lactol **132** in CH_2Cl_2 (5 mL). After 18 h, the solvent was removed under reduced pressure and the ensuing residue was purified by flash chromatography (silica; hexane) to provide the title compound as a pale-yellow oil (10 mg, 0.046 mmol, 13% yield over two steps). The spectroscopic data obtained was consistent with data reported in the literature.[288]

^{1}H NMR (400 MHz, $CDCl_3$): δ	7.08 (d, *J* = 1.5 Hz, 1H), 7.05 (q, *J* = 1.5 Hz, 1H), 2.77 (dd, *J* = 16.4, 6.4 Hz, 1H), 2.50 (dddd, *J* = 16.4, 12.0, 7.4, 1.8 Hz, 1H), 1.97 (m, 1H), 1.81 (dd, *J* = 13.5, 7.4 Hz, 1H), 1.74–1.44 (complex m, 5H), 1.30–1.24 (complex m, 2H), 1.21 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	137.6, 137.1, 134.9, 120.1, 51.7, 42.2, 39.6, 34.0, 33.7, 33.3, 25.1, 21.7, 20.6, 19.24, 19.18.
$[\alpha]_D^{22}$	+17 (c 0.2, $CHCl_3$). Lit. $[\alpha]_D$ +19 (c 1.0, $CHCl_3$).[288]

(5a*S*,9*R*,9a*S*)-6,6,9a-trimethyl-3,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-*c*]furan-9-yl acetate, (5a*S*,9*R*,9a*S*)-6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-*c*]furan-9-yl acetate

To a solution of 1β-acetoxyisodrimeninol (**81**) (200 mg, 0.68 mmol) in CH_2Cl_2 (10 mL) was added HCl (10 drops, 2 M). The reaction was stirred for 18 h before being evaporated to dryness. Purification via automated gradient flash column chromatography (silica; 0 → 100 % EtOAc/hexane) provided furan **135** (25 mg, 0.090 mmol, 13%), intermediate **134** (14 mg, 0.051 mmol, 7.4%), and recovered starting material (**81**) (84 mg, 0.29 mmol, 42% yield).

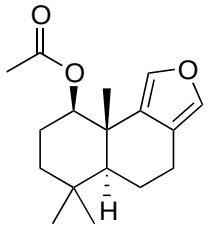
(5a*S*,9*R*,9a*S*)-6,6,9a-trimethyl-3,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-*c*]furan-9-yl acetate



134

HRMS	Found M^+ 276.1723, $C_{17}H_{24}O_3$ requires M^+ 276.1725.
ν_{max} (NaCl):	2953, 2922, 2968, 2853, 1740 (C=O), 1369, 1240, 1032, 1010, 964, 922 cm^{-1}.
^{1}H NMR (600 MHz, $CDCl_3$): δ	5.60 (d, *J* = 1.7 Hz, 1H), 5.54 (m, 1H), 4.54 (dd, *J* = 11.3, 4.5 Hz, 1H), 4.12 (m, 1H), 4.02 (d, *J* = 10.9 Hz, 1H), 2.32 (s, 1H), 2.22 (m, 1H), 2.14 (s, 3H), 1.99 (m, 1H), 1.91 (dq, *J* = 12.9, 4.3 Hz, 1H), 1.56 (m, 1H), 1.44 (m, 2H), 1.38 (m, 1H), 0.91 (s, 3H), 0.88 (s, 3H), 0.86 (2, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	171.1, 136.4, 116.6, 100.8, 81.6, 68.0, 60.3, 49.0, 39.6, 37.6, 32.8, 32.1, 24.0, 23.9, 22.2, 20.8, 9.9.
MS (EI) m/z	276 (M^+), 219, 201, 175, 145, 133, 87, 81.
$[\alpha]_D^{20}$	−121 (c 0.75, $CHCl_3$).

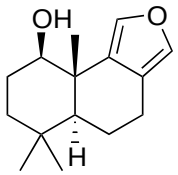
(5a*S*,9*R*,9a*S*)-6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-*c*]furan-9-yl acetate



135

HRMS	Found M^+ 276.1731, $C_{17}H_{24}O_3$ requires M^+ 276.1725.
ν_{max} (NaCl):	2953, 2925, 2871, 2851, 1735 (C=O), 1381, 1374, 1239, 1044, 1038, 1030 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	7.09 (d, *J* = 1.3 Hz, 1H), 7.07 (m, 1H), 4.74 (dd, *J* = 11.5, 4.1 Hz, 1H), 2.77 (dd, *J* = 16.2, 5.9 Hz, 1H), 2.48 (m, 1H), 2.17 (s, 3H), 1.89 (dq, *J* = 13.0, 3.6 Hz, 1H), 1.81 (ddt, *J* = 13.4, 7.0, 1.5 Hz, 1H), 1.79–1.66 (complex m, 2H), 1.54 (dt, *J* = 13.7, 3.7 Hz, 1H), 1.48 (td, *J* = 13.6, 4.0 Hz), 1.35 (dd, *J* = 12.0, 1.7 Hz, 1H), 1.32 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	170.5, 136.7, 136.0, 133.3, 119.9, 81.2, 51.4, 39.6, 38.5, 33.0, 32.8, 24.3, 21.7, 21.6, 20.8, 19.7, 19.0.
MS (EI) m/z	276 (M+, 50), 217 (35), 201 (24) 175 (35), 145 (22), 133 (25), 119 (29), 105 (18), 87 (37), 81 (22).
$[\alpha]_D^{20}$	+10 (c 0.5, $CHCl_3$).

(5a*S*,9*R*,9a*S*)-6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-*c*]furan-9-ol

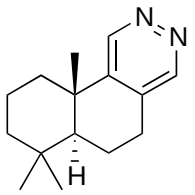


136

To a solution of acetoxyeuryfuran (**135**) (10 mg, 0.036 mmol) in dry THF (10 mL) at 0 °C under N_2 was added $LiAlH_4$ (50 mg, 1.32 mmol, excess). The reaction was warmed to rt and stirred for 30 min water (150 µL) was added to quench the excess reagent. The reaction was diluted with CH_2Cl_2 (20 mL), dried ($MgSO_4$), filtered and evaporated to yield a colourless oil (12 mg). Purification via automated gradient flash column chromatography (silica; 0 → 30 % EtOAc/hexane) provided the title compound (5 mg, 0.021 mmol, 58% yield).

HRMS	Found $M+H^+$ 235.1693, $C_{15}H_{23}O_2$ requires M^+ 235.1693.
ν_{max} (NaCl):	3420 (br, -OH), 2949, 2928, 1458, 1389, 1368, 1084, 1040, 1027, 988, 888, 789 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	7.57 (d, *J* = 1.5 Hz, 1H), 7.08 (m, 1H), 3.63 (dd, *J* = 11.5, 4.4 Hz, 1H), 2.79 (dd, *J* = 16.2, 6.1 Hz, 1H), 2.49 (m, 1H), 1.84–1.68 (complex m, 4H), 1.53 (dt, *J* = 13.8, 3.5 Hz, 1H), 1.40 (td, *J* = 13.7, 4.3 Hz, 1H), 1.28 (s, 1H), 1.26 (dd, *J* = 11.9, 2.0 Hz, 1H), 1.23, (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	137.5, 136.3, 134.2, 120.0, 78.5, 51.1, 40.1, 39.9, 33.0, 32.7, 29.0, 21.5, 20.7, 19.1, 18.2.
$[\alpha]_D^{20}$	negligible (c 0.2, $CHCl_3$).

(6a*S*,10a*S*)-7,7,10a-Trimethyl-5,6,6a,7,8,9,10,10a-octahydrobenzo[*f*]phthalazine



137

Hydrazine hydrate (150 mg, 3.00 mmol) was added to a solution of polygodial (**12**) (52 mg, 0.22 mmol) in EtOH (10 mL). The reaction mixture was heated at reflux for 1.5 h then cooled and the mixture was concentrated under reduced pressure to provide the title compound as a colourless solid (51 mg, 0.22 mmol, >99% yield).

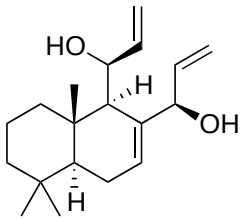
HRMS	Found M^+ 230.1789, $C_{15}H_{22}N_2$ requires M^+ 230.1783.
ν_{max} (NaCl):	2999, 2959, 2947, 2926, 2866, 1559, 1458, 1422, 1383, 1175, 997, 909, 733 cm^{-1}.
^{1}H NMR (400 MHz, $CDCl_3$): δ	8.99 (s, 1H), 8.80 (s, 1H), 2.91 (dd, *J* = 18.5, 6.7 Hz, 1H), 2.80 (ddd, *J* = 18.5, 11.1, 7.5 Hz, 1H), 2.34 (d, *J* = 12.5 Hz, 1H), 2.00 (dd, *J* = 13.7, 7.5 Hz, 1H), 1.85–1.62 (complex m, 3H), 1.53 (m, 1H), 1.41 (td, *J* = 13.0, 4.0 Hz, 1H) 1.30–1.19 (complex m, 5H), 0.97 (s, 3H), 0.94 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	152.3, 149.1, 148.4, 135.7, 49.6, 41.4, 37.1, 36.5, 33.6, 33.2, 27.0, 24.3, 21.6, 18.8, 17.9.
MS (EI) m/z	230 (M^+), 215, 161, 159, 147, 145, 133, 119, 108, 91.
$[\alpha]_D^{22}$	+17 (c 0.7, $CHCl_3$).

(1*S*,1'*R*)-1,1'-((1*R*,4a*S*,8a*S*)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1,2-diyl)bis(prop-2-en-1-ol),
(1*S*,1'*S*)-1,1'-((1*R*,4a*S*,8a*S*)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1,2-diyl)bis(prop-2-en-1-ol)

To a solution of polygodial (**12**) (300 mg, 1.28 mmol) in dry THF (5 mL) at 0 °C under N_2 was added vinylmagnesium bromide (2.0 mL, 0.8 M in THF, 1.6 mmol, excess). The reaction was stirred for 72 h before sat. NH_4Cl was added (10 mL) and the reaction

extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield a crude orange oil (447 mg). Purification via automated gradient flash column chromatography (silica; 0 → 20 % EtOAc/hexane) provided the title compounds in a total yield of 324 mg (0.113 mmol, 86 % yield). Clean fractions were combined to provide isomer **138** as a white amorphous solid (107 mg, 0.37 mmol) and isomer **139** as a white crystalline solid (174 mg, 0.61 mmol).

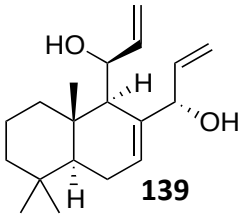
(1*S*,1'*R*)-1,1'-((1*R*,4a*S*,8a*S*)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1,2-diyl)bis(prop-2-en-1-ol)



138

HRMS	Found $[M+Na]^+$ 313.2139, $C_{19}H_{30}O_2Na$ requires M^+ 313.2138.
ν_{max} (NaCl):	3269 (br, -OH), 3082, 2947, 2923, 2865, 2847, 1456, 1389, 1133, 1112, 988, 915, 842, 758 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	6.20 (ddd, *J* = 17.3, 10.7, 4.1 Hz, 1H), 6.04 (ddd, *J* = 17.3, 10.6, 4.8 Hz, 1H), 5.99 (dt, *J* = 6.8, 3.2 Hz, 1H), 5.36–5.28 (complex m, 2H), 5.20 (dt, *J* = 10.6, 1.6 Hz, 1H), 5.18 (ddd, *J* = 10.7, 2.3, 1.5 Hz, 1H), 4.94 (d, *J* = 4.6 Hz, 1H), 4.60 (m, 1H), 2.38 (m, 1H), 2.04 (m, 2H), 1.95 (m, 1H), 1.60 (qt, *J* = 13.8, 3.2 Hz, 1H), 1.53–1.43 (complex m, 2H), 1.23–1.15 (complex m, 2H), 1.08–1.01 (complex m, 4H), 0.94 (s, 3H), 0.89 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	144.7, 139.7, 137.9, 129.9, 114.4, 112.8, 74.3, 69.5, 57.2, 49.6, 42.0, 40.3, 37.1, 33.3, 32.9, 23.4, 22.3, 18.7, 14.9.
$[\alpha]_D^{20}$	+2.0 (c 1.1, $CHCl_3$).

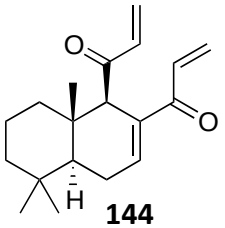
(1*S*,1'*S*)-1,1'-((1*R*,4a*S*,8a*S*)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1,2-diyl)bis(prop-2-en-1-ol)



139

HRMS	Found $[M+Na]^+$ 313.2140, $C_{19}H_{30}O_2Na$, requires M^+ 313.2138.
ν_{max} (NaCl):	3261 (br, -OH), 2981, 2948, 2923, 2865, 2846, 1457, 1441, 1389, 1367, 1134, 1111, 987, 918, 756 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	6.10 (ddd, *J* = 17.2, 10.7, 3.3 Hz, 1H), 6.04 (dt, *J* = 6.4, 2.4 Hz, 1H), 5.90 (ddd, *J* = 17.2, 10.6, 4.5 Hz, 1H), 5.40 (dq, *J* = 17.2, 1.4 Hz, 1H), 5.30 (dt, *J* = 17.3, 1.7 Hz, 1H), 5.16–5.11 (complex m, 2H), 4.84 (m, 1H), 4.61 (q, *J* = 3 Hz, 1H), 2.20 (m, 1H), 2.09 (m, 1H), 2.02–1.94 (complex m, 2H), 1.63 (qt, *J* = 13.7, 3.1 Hz, 1H), 1.53–1.44 (complex m, 2H), 1.22–1.15 (complex m, 2H), 1.07 (s, 3H), 1.02 (td, *J* = 13.1, 3.5 Hz, 1H), 0.93 (s, 3H), 0.90 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	144.9, 140.0, 137.1, 131.0, 112.8, 112.1, 70.8, 69.4, 60.2, 49.8, 42.1, 40.2, 37.3, 33.7, 32.9, 23.4, 22.2, 18.8, 15.1.
$[\alpha]_D^{20}$	−64 (c 1.6, $CHCl_3$).

1,1'-((1*R*,4a*S*,8a*S*)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalene-1,2-diyl)bis(prop-2-en-1-one)



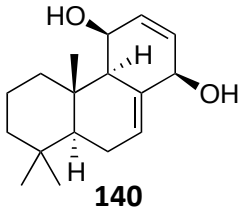
144

To a mixture of isomers **138** and **139** (98 mg, 0.34 mmol) at 0 °C in CH_2Cl_2 was added Dess-Martin periodinane (400 mg, 0.95 mmol) slowly. The reaction was warmed to rt and stirred for 15 min. Hexanes (15 mL) was added and the crude reaction mixture filtered on a silica plug eluting with 30 % EtOAc:hexanes to yield a colourless oil (105 mg). Purification via automated gradient flash column chromatography (silica; 0 → 20 % EtOAc/hexane) provided the title compound as a colourless oil (50 mg, 0.17 mmol, 50% yield)

HRMS	Found M^+ 286.1933, $C_{19}H_{26}O_2$ requires M^+ 283.1933.
ν_{max} (NaCl):	2926, 2867, 2848, 1693, 1656, 1645, 1608, 1458, 1408, 1398, 1366, 1269, 1227, 1197, 1143, 1088, 979, 961, 764 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	7.02 (dt, *J* = 5.8, 2.2 Hz, 1H), 6.85 (dd, *J* = 17.1, 10.6 Hz, 1H), 6.55 (17.2, 10.4 Hz, 1H), 6.26–6.22 (complex m, 2H), 5.74 (dd, *J* = 10.6, 1.8 Hz, 1H), 5.66 (dd, *J* = 10.5, 1.4 Hz, 1H), 3.72 (m, 1H), 2.35 (m, 1H), 2.26 (dddd, *J* = 19.5, 12.0, 3.8, 2.6 Hz, 1H), 1.76 (m, 1H), 1.60–1.41 (complex m, 4H), 1.34 (dd, *J* = 12.0, 4.7 Hz, 1H), 1.26 (m, 1H), 0.97 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	200.8, 191.5, 141.8, 140.3, 137.9, 131.6, 128.8, 126.5, 61.1, 49.0, 41.8, 41.4, 36.6, 33.3, 33.0, 24.5, 22.0, 18.4, 15.0.

MS (EI) m/z	286 (M^+), 232, 217, 199, 175, 163, 162, 161, 149, 135, 120, 118, 109, 95, 87, 84, 82.
$[\alpha]_D^{20}$	+125 (c 0.8, $CHCl_3$).

(1*R*,4*S*,4a*R*,4b*S*,8a*S*)-4b,8,8-trimethyl-1,4,4a,4b,5,6,7,8,8a,9-decahydrophenanthrene-1,4-diol



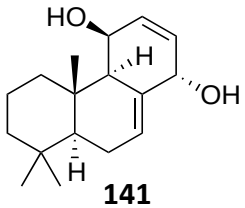
140

To a solution of diol **138** (105 mg, 0.36 mmol) in dry CH_2Cl_2 (25 mL) was added Grubbs Catalyst™ C848 (Grubbs 2nd generation catalyst, CAS no. 246047-72-3) (25 mg, 0.029 mmol, 8%) slowly. The reaction was stirred for 10 min before the solvent was removed under reduced pressure to yield a brown semi-solid material (130 mg). Purification via automated gradient flash column chromatography (silica; 0 → 70 % EtOAc/hexane) provided the title compound as an amorphous white solid (71 mg, 0.34 mmol, 94% yield)

HRMS	Found M^+ 262.1928, $C_{17}H_{26}O_2$ requires M^+ 262.1933.
ν_{max} (NaCl):	3314 (br, -OH), 3040, 2922, 2844, 1456, 1443, 1388, 1366, 1114, 1096, 975, 918, 837, 756 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	6.55 (dd, *J* = 9.4, 6.6 Hz, 1H), 6.22 (dd, *J* = 9.4, 5.9 Hz, 1H), 5.99 (m, 1H), 4.54 (d, *J* = 5.4 Hz, 1H), 4.41 (t, *J* = 5.9 Hz, 1H), 2.82 (S, broad -OH, 2H), 2.22 (m, 1H), 2.10–1.97 (complex m, 3H), 1.67 (qt, *J* = 13.7, 3.2 Hz, 1H), 1.53 (d quint, *J* = 13.7, 3.6 Hz, 1H), 1.47 (m, 1H), 1.26 (dd, *J* = 12.2, 5.1 Hz, 1H), 1.22–1.09 (complex m, 5H), 0.95 (s, 3H), 0.87 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	138.4, 135.1, 131.6, 129.5, 67.1, 63.5, 56.0, 50.6, 42.3, 38.9, 36.1, 33.4, 32.9, 24.5, 21.5, 18.7, 15.4.
MS (EI) m/z	262 (M^+), 244, 226, 211, 173, 159, 155, 141, 131, 121, 120, 109, 107, 91, 87, 81.

$[\alpha]_D^{20}$ +43 (c 0.8, $CHCl_3$).

(1*S*,4*S*,4a*R*,4b*S*,8a*S*)-4b,8,8-trimethyl-1,4,4a,4b,5,6,7,8,8a,9-decahydrophenanthrene-1,4-diol



141

To a solution of diol **139** (96 mg, 0.33 mmol) in dry CH_2Cl_2 (25 mL) was added Grubbs Catalyst™ C848 (Grubbs 2nd generation catalyst, CAS no. 246047-72-3) (25 mg, 0.029 mmol, 9%) slowly. The reaction was stirred for 10 min before the solvent was removed under reduced pressure to yield a brown semi-solid material (120 mg). Purification via automated gradient flash column chromatography (silica; 0 → 70 % EtOAc/hexane) provided the title compound as an amorphous white solid (60 mg, 0.29 mmol, 88% yield)

HRMS Found M^+ 262.1934, $C_{17}H_{26}O_2$ requires M^+ 262.1933.

ν_{max} (NaCl): 3437, 3293, 2962, 2925, 2848, 1396, 1070, 1056, 1028, 989, 965, 930, 833, 789, 779 cm^{-1}.

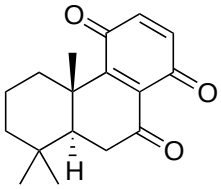
^{1}H NMR (600 MHz, $CDCl_3$): δ 6.25 (ddd, *J* = 9.7, 6.4, 2.2 Hz, 1H), 6.19 (m, 1H), 5.91 (dd, *J* = 9.8, 2.4 Hz, 1H), 4.77 (s, 1H), 4.43 (s, 1H), 2.20–2.06 (complex m, 3H), 2.02 (m, 1H), 1.66 (qt, *J* = 13.6, 3.0 Hz, 1H), 1.53–1.45 (complex m, 2H), 1.30–1.10 (complex m, 6H), 0.96 (s, 3H), 0.89 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 136.3, 133.2, 132.0, 128.5, 66.8, 63.2, 53.4, 50.7, 42.1, 40.2, 36.1, 33.6, 32.9, 23.9, 22.1, 18.6, 16.3.

MS (EI) m/z 262 (M^+), 244, 226, 211, 173, 155, 141, 124, 123, 121, 120, 109, 107, 87, 82.

$[\alpha]_D^{20}$ +210 (c 0.6, $CHCl_3$).

(4b*S*,8a*S*)-4b,8,8-trimethyl-5,6,7,8,8a,9-hexahydrophenanthrene-1,4,10(4b*H*)-trione



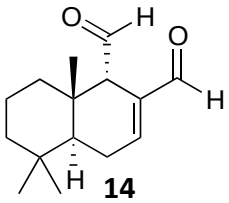
143

To a solution of diol **141** (71 mg, 0.27 mmol) in CH_2Cl_2 (15 mL) was added Dess-Martin periodinane (290 mg, 0.81 mmol) slowly. The reaction was stirred for 3 h before being diluted with hexane (30 mL) and filtered through a silica plug eluting with 30% EtOAc:hexane (100 mL). The solvent was removed under reduced pressure to yield a yellow oil (90 mg) Fractionation via automated gradient flash column chromatography yielded a quinone containing fraction. This fraction was redissolved in CH_2Cl_2 (5 mL) and Dess-Martin periodinane (50 mg, 0.14 mmol) was added and the resulting yellow mixture stirred for 1 h. Sodium metabisulfite (sat. aq., 5 mL) was added, and the organic fraction separated. The aqueous mixture was extracted with CH_2Cl_2 (2 x 5 mL), before the combined organic fractions were washed with Na_2CO_3 (sat. aq., 2 x 10 mL). The organic phase was then dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to yield a yellow oil (10 mg). Purification via automated gradient flash column chromatography (silica; 0 → 20 % EtOAc/hexane) provided the title compound as an amorphous pink semi-solid (2 mg, 0.0073 mmol, 3% yield)

HRMS	Found M^+ 272.1413, $C_{17}H_{20}O_3$ requires M^+ 272.1412.
ν_{max} (NaCl):	2926, 2866, 1704, 1660, 1653, 1329, 1278, 1124, 1098, 841 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	6.71 (d, *J* = 10.1 Hz, 1H), 6.67 (d, *J* = 10.1 Hz, 1H), 2.78 (m, 1H), 2.70 (dd, *J* = 18.1, 3.8 Hz, 1H), 2.56 (dd, *J* = 18.1, 4.7 Hz, 1H), 1.85–1.74 (complex m, 2H), 1.63 (m, 1H), 1.53 (m, 1H), 1.43 (s, 3H), 1.30–1.24 (complex m, 2H), 0.99 (s, 3H), 0.96 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	197.0, 188.6, 184.7, 160.5, 137.3, 135.5, 130.8, 49.1, 40.7, 40.2, 36.6, 35.6, 33.3, 32.7, 21.3, 18.5, 18.0.

MS (EI) m/z	272 (M^+), 257, 239, 229, 215, 190, 189, 177, 176, 175, 164, 163, 109, 95, 87, 77.
$[\alpha]_D^{20}$	−704 (c 0.01, $CHCl_3$).

9-Epipolygodial

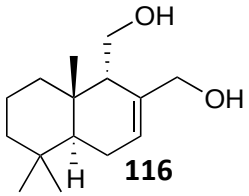


14

Polygodial (**12**) (300 mg) and K_2CO_3 (40 mg) were suspended in EtOH/H_2O (5 mL of a 20% EtOH/H_2O solution) in a 10 mL sealed microwave reactor tube. The resulting suspension was stirred at 110 °C in a microwave reactor for 50 min. H_2O (15 mL) was added and the resulting mixture extracted with CH_2Cl_2 (3 x 5 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide a yellow oil. Purification via automated gradient flash chromatography (silica; 0 → 20 % EtOAc/hexane) provided the title compound as a white amorphous solid, (**14**, 110 mg, 37% yield) and recovered starting material polygodial (**12**, 68 mg, 23% yield). The spectroscopic data obtained was consistent with data reported in the literature.[240]

^{1}H NMR (400 MHz, $CDCl_3$) δ	9.82 (d, *J* 2.6, 1H), 9.38 (s, 1H), 7.07 (dd, *J* 4.9, 2.6, 1H), 3.22 (m, 1H), 2.54 (dt, *J* 20.6, 5.0, 1H) 2.19 (ddt, *J* 20.6, 11.7, 2.1), 1.75 (m, 1H), 1.67–1.37 (complex m, 5H), 1.13 (td, *J* 13.2, 3.6, 1H), 0.93 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$) δ	202.3, 192.9, 153.6, 137.4, 58.5, 44.3, 42.1, 37.7, 37.1, 32.9, 32.8, 25.6, 21.9, 21.5, 18.4.
$[\alpha]_D^{20}$	−436 (c = 0.55, 95% EtOH). −428 (c = 0.5, $CHCl_3$) lit. $[\alpha]_D$ −237 (95% EtOH)[327]

9-Epidrimendiol



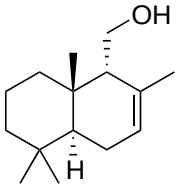
116

To a solution of 9-epipolygodial (**14**) (103 mg, 0.44 mmol) in EtOH (10 mL) at 0°C was added $NaBH_4$ (63 mg, 1.67 mmol). The reaction was stirred at 0 °C for 5 min and rt for 2 h. H_2O (40 mL) was added, and the reaction extracted with CH_2Cl_2 (3 x 20 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to provide the title compound as a white amorphous solid (102 mg, 0.43 mmol, 98% yield) used without further purification. The spectroscopic data obtained was consistent with data reported in the literature.[273]

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.84 (t, *J* = 3.5 Hz, 1H), 4.14 (d, *J* = 12.0 Hz, 1H), 4.01 (d, *J* = 12.0 Hz, 1H), 3.94 (dd, *J* = 11.0, 4.5 Hz, 1H), 3.64 (dd, *J* = 11.0, 5.9 Hz, 1H), 2.99 (br s, 2H), 2.16 (dt, *J* = 18.8, 5.1 Hz, 1H), 1.92 (m, 1H), 1.78 (m, 1H), 1.72–1.33 (complex m, 7H), 0.92 (br s, 6H), 0.88 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 136.9, 127.8, 67.7, 63.2, 54.0, 43.3, 42.7, 36.5, 36.0, 33.1, 33.0, 24.3, 22.0, 21.7, 18.8.

9-Epidrimenol



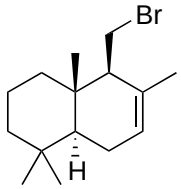
117

A solution of 9-epidrimendiol (**116**) (54 mg, 0.23 mmol) in EtOH (15 mL) was subjected to a hydrogen atmosphere (1 atm) with catalytic Pd/C. The reaction was stirred at rt for 1 h before being filtered through Celite™ and evaporated to dryness to yield a colourless oil. Purification via automated gradient flash column chromatography (silica; 0 → 20 %

EtOAc/hexane) provided the title compound as a white amorphous solid (33 mg, 0.15 mmol, 65 % yield). The spectroscopic data obtained was consistent with data reported in the literature.[273]

^{1}H NMR (400 MHz, $CDCl_3$): δ	5.57 (br s, 1H), 3.74 (m, 2H), 2.02 (m, 1H), 1.84 (m, 1H), 1.74 (s, 3H), 1.68–1.57 (complex m, 2H), 1.57–1.41 (complex m, 4H), 1.37–1.20 (complex m, 2H), 0.92 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	131.4, 124.6, 61.3, 57.6, 43.5, 42.7, 36.8, 36.1, 33.2, 33.0, 24.0, 23.0, 22.2, 21.7, 18.8.

(4a*S*,5*S*,8a*S*)-5-(bromomethyl)-1,1,4a,6-tetramethyl-1,2,3,4,4a,5,8,8a-octahydronaphthalene



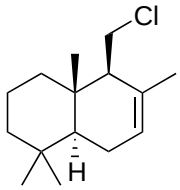
158

To (–)-drimenol (**118**) (72 mg, 0.32 mmol) and PPh_3 (119 mg, 0.45 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added *N*-bromosuccinimide (83 mg, 0.47 mmol). The reaction was stirred at rt for 1 h before the solvent was removed under reduced pressure to yield a crude white semi-solid. Filtration through a short silica plug eluting with hexanes and subsequent evaporation of the solvent provided a crude colourless oil (50 mg). Purification via flash column chromatography (silica; hexane) provided the title compound as a colourless oil (10 mg, 0.035 mmol, 11% yield)

HRMS	Found $[M–H]^+$ 283.1059, $C_{15}H_{24}{}^{79}Br$ requires M^+, 240.1061.
ν_{max} (NaCl):	2959, 2924, 1456, 1387, 1366, 1271, 1229, 1217, 1206, 1074 cm^{-1}.
^{1}H NMR (600 MHz, $CDCl_3$): δ	4.49 (m, 1H), 3.66 (dd, *J* = 10.8, 2.1 Hz, 1H), 3.29 (dd, *J* = 10.8, 6.7 Hz, 1H), 2.37 (m, 1H), 2.05–1.79 (complex m, 6H), 1.63–1.39 (complex m, 3H), 1.23–

	1.10 (complex m, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	132.6, 123.9, 57.7, 50.0, 42.0, 39.3, 37.7, 33.2, 33.0, 31.4, 23.6, 21.9, 21.7, 18.7, 13.9.
MS (EI) m/z	285 ([^{81}Br–H]-M^+), 283 ([^{79}Br–H]-M^+), 221, 205, 191, 177, 153, 147, 135, 123, 109, 107, 95, 81, 69, 55, 43, 41.
$[\alpha]_D^{20}$	+34 (c 0.5, $CHCl_3$).

(4a*S*,5*S*,8a*S*)-5-(chloromethyl)-1,1,4a,6-tetramethyl-1,2,3,4,4a,5,8,8a-octahydronaphthalene



159

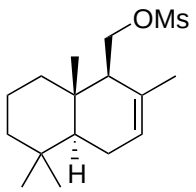
To (–)-drimenol (**118**) (180 mg, 0.81 mmol) in CCl_4 (15 mL) was added pyridine (3 mL) and triphenylphosphine (1.0 g, 3.81 mmol, excess). The reaction was stirred at reflux for 1 h before the solvent was removed under reduced pressure to yield a crude white semi-solid. Filtration through a short silica plug eluting with 50% CH_2Cl_2:hexanes provided a colourless oil (450 mg). Purification via automated gradient flash column chromatography (silica; 0 → 10 % EtOAc/hexane) provided the title compound as a colourless oil (80 mg, 0.33 mmol, 41% yield).

HRMS	Found M^+ 240.1648, $C_{15}H_{25}{}^{35}Cl$ requires M^+, 240.1645.
ν_{max} (NaCl):	2963, 2945, 2923, 2905, 2865, 2847, 1456, 1442, 1389, 1366, 750 cm^{-1}.
^{1}H NMR (600 MHz, $CDCl_3$): δ	5.53 (m, 1H) 3.78 (dd, *J* = 11.6, 2.5 Hz, 1H), 3.50 (dd, *J* = 11.6, 6.4, 1H), 2.23 (s, 1H), 2.03 (m, 1H), 1.96 (m, 1H), 1.89 (m, 1H), 1.84 (m, 3H), 1.59 (qt, *J* = 13.6, 3.1

Hz, 1H), 1.51 (d quint, *J* = 13.9, 3.6 Hz, 1H), 1.45 (m, 1H), 1.25–1.17 (complex m, 2H), 1.15 (td, *J* = 13.1, 3.9 Hz, 1H), 0.91 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ	132.4, 124.0, 57.5, 50.0, 43.1, 42.0, 39.5, 37.1, 33.2, 33.0, 23.6, 21.9, 21.7, 18.7, 14.3.
MS (EI) m/z	240 (^{35}Cl-M^+), 225, 147, 135, 125, 124, 123, 122, 120, 110, 109, 107, 105, 97, 96, 95, 93, 91, 87, 82, 81, 79, 77.
$[\alpha]_D^{20}$	negligible (c 1.0, $CHCl_3$).

((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl methanesulfonate

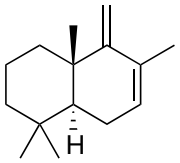


160

To (–)-drimenol (**118**) (100 mg, 0.45 mmol) in CH_2Cl_2 (10 mL) was added methanesulfonyl chloride (42 µL, 62 mg, 0.54 mmol). The reaction was stirred at rt for 1 h before HCl (2 M, 20 mL) was added. The organic layer was then separated and washed with sat. Na_2CO_3 and HCl (2 M) before being dried ($MgSO_4$), filtered and evaporated to provide the title compound as a yellow oil (149 mg) used without further purification. The spectroscopic data obtained was consistent with data reported in the literature.[328]

^{1}H NMR (600 MHz, $CDCl_3$): δ	5.56 (m, 1H), 4.44 (dd, *J* = 10.0, 3.2 Hz, 1H), 4.26 (dd, *J* = 10.0, 6.1 Hz, 1H), 3.02 (s, 3H), 2.16 (s, 1H), 2.04 (m, 1H), 1.97 (m, 1H), 1.89 (m, 1H), 1.76 (m, 3H), 1.63–1.48 (complex m, 2H), 1.46 (m, 1H), 1.26–1.12 (complex m, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	131.2, 124.6, 68.1, 53.8, 49.7, 41.9, 39.6, 37.5, 36.1, 33.3, 33.0, 23.5, 21.9, 21.7, 18.6, 14.6.

(4a*S*,8a*S*)-1,1,4a,6-tetramethyl-5-methylene-1,2,3,4,4a,5,8,8a-octahydronaphthalene



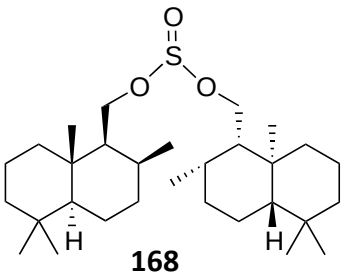
157

To crude drimenyl mesylate **160** (149 mg) in dry THF (10 mL) under N_2 was added KO*t*Bu (103 mg, 0.92 mmol). The reaction mixture was stirred at rt for 3 h and 40 °C for 30 min. A further portion of KO*t*Bu (100 mg, 0.89 mmol) was added and the reaction stirred at 40 °C for a further 2.5 h. CH_2Cl_2 (50 mL) and H_2O (50 mL) were added, the layers were separated, and the aqueous portion extracted with CH_2Cl_2 (2 x 15 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to provide the title compound as a colourless oil (66 mg, 0.32 mmol, 71% yield over 2 steps). Used without further purification. The spectroscopic data obtained was consistent with data reported in the literature.[329]

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.69 (br s, 1H), 4.83 (d, *J* = 16.4 Hz, 2H), 2.22–1.98 (complex m, 2H), 1.90 (d, *J* = 12.2 Hz, 1H) 1.82 (s, 3H), 1.73–1.13 (complex m, 6H), 0.99 (s, 3H), 0.95 (s, 3H), 0.89 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 158.2, 131.2, 126.5, 103.7, 48.7, 42.2, 37.8, 37.7, 33.4, 32.9, 24.3, 22.1, 21.1, 20.6, 19.1.

bis(((1*S*,2*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)methyl) sulfite



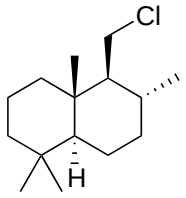
168

Syn-drimanol **120** (70 mg, 0.31 mmol) in 50% $SOCl_2$:CH_2Cl_2 (6 mL) was stirred at rt for 20 h before the solvent was removed under reduced pressure to yield a crude oil (70 mg). Purification via automated gradient flash column chromatography (silica; 0 → 25 %

EtOAc/hexane) provided the title compound as a colourless crystalline solid (42 mg, 0.085 mmol (0.17 mmol equiv of drimanol), 55 % yield) and recovered starting material (22 mg, 0.097 mmol, 31 % recovered).

HRMS	Found $[M+Na]^+$ 517.3690, $C_{30}H_{53}O_3NaS$ requires M^+ 517.3686.
ν_{max} (NaCl):	2922, 2867, 2851, 1465, 1457, 1387, 1366, 1210, 1200, 1191, 956, 949, 930, 920, 755, 705 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	4.25 (dd, *J* = 10.1, 4.5 Hz, 1H), 4.18 (dd, *J* = 10.1, 4.5 Hz, 1H), 3.99 (t, *J* = 9.9 Hz, 1H), 3.88 (t, *J* = 9.9 Hz, 1H), 2.15 (m, 2H), 1.72–1.66 (complex m, 4H), 1.65–1.49 (complex m, 8H), 1.46–1.36 (complex m, 6H), 1.19 (td, *J* = 13.3, 3.7 Hz, 2H), 1.06 (tt, *J* = 12.8, 4.2 Hz, 2H), 0.99 (dd, *J* = 7.5, 3.9 Hz, 6H), 0.92–0.88 (complex m, 14H), 0.84 (s, 6H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	61.5, 60.7, 56.5, 56.4, 52.7, 52.6, 41.92, 41.90, 39.91, 39.84, 37.7 (2C), 34.21, 34.19, 33.54, 33.53, 33.28, 33.27, 28.85, 28.80, 21.6 (2C), 18.33, 18.31, 17.40, 17.39, 17.06, 17.04, 15.64, 15.60.
$[\alpha]_D^{20}$	+31 (c 1.15, $CHCl_3$).

(4a*S*,5*S*,6*R*,8a*S*)-5-(chloromethyl)-1,1,4a,6-tetramethyldecahydronaphthalene



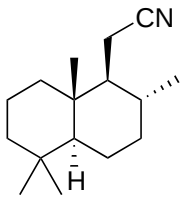
169

To *anti*-drimanol (**119**) (200 mg, 0.89 mmol) in CCl_4 (15 mL) was added pyridine (5 mL) and triphenylphosphine (1.2g, 4.56 mmol, excess). The reaction was stirred at reflux for 50 min before the solvent was removed under reduced pressure to yield a crude white

semi-solid. Purification via a short silica plug eluting with hexanes provided the title compound as a colourless oil (155 mg, 0.64 mmol, 72 % yield)

HRMS	Found M^+ 242.1804, $C_{15}H_{27}{}^{35}Cl$ requires M^+ 242.1801.
ν_{max} (NaCl):	2924, 2868, 2846, 1464, 1442, 1389, 1368, 1302, 1282, 1114, 1035, 975, 743, 630 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ	3.68 (dd, *J* = 11.8, 3.3 Hz, 1H), 3.50 (dd, *J* = 11.8, 3.3, 1H), 1.89–1.78 (complex m, 2H), 1.73–1.55 (complex m, 3H), 1.49 (d quint, *J* = 13.9, 3.6 Hz, 1H), 1.42 (m 1H), 1.38–1.25 (complex m, 2H), 1.17 (td, *J* = 13.3, 4.0 Hz, 1H), 1.12–1.03 (complex m, 3H), 1.01 (d, *J* = 6.4 Hz, 3H), 0.92 (s, 3H), 0.90–0.83 (complex m, 7H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	60.0, 55.1, 43.7, 41.9, 39.3, 38.4, 36.5, 33.6, 33.3, 31.6, 21.8, 21.7, 20.7, 18.7, 15.1.
MS (EI) m/z	242 (^{35}Cl-M^+), 229, 227, 137, 124, 123, 122, 121, 120, 119, 118, 117, 109, 95.
$[\alpha]_D^{20}$	−6.5 (c 0.65, $CHCl_3$).

2-((1*S*,2*R*,4a*S*,8a*R*)-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)acetonitrile



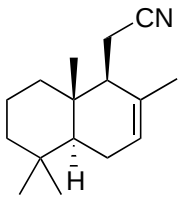
170

To a solution of *anti*-drimanyl chloride (**169**) (161 mg, 0.66 mmol) in DMF (20 mL) was added KCN (740 mg, 11.4 mmol, excess). The reaction was heated to 70 °C and stirred for 18 h. Water (30 mL) was added and the reaction extracted with toluene (3 x 20 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield the crude product as a colourless oil (75 mg). Purification via automated gradient flash

column chromatography (silica; 0 → 15 % EtOAc/hexane) provided the title compound as a colourless oil (15 mg, 0.064 mmol, 10% yield) and recovered starting material (42 mg, 0.17 mmol, 26 % yield).

HRMS	Found M^+ 233.2141, $C_{16}H_{27}N$ requires M^+, 233.2143.
ν_{max} (NaCl):	2926, 2869, 2846, 2242 (CN), 1463, 1443, 1426, 1389, 1369, 1205, 1116, 1036, 992, 976 cm^{-1}.
^1H NMR (600 MHz, $CDCl_3$): δ	2.41 (dd, *J* = 17.6, 5.0 Hz, 1H), 2.25 (dd, *J* = 17.6, 4.5 Hz, 1H), 1.84 (dq, *J* = 13.3, 3.1 Hz, 1H), 1.73 (m, 1H), 1.67 (m, 1H), 1.65–1.56 (complex m, 2H), 1.50 (d quint, *J* = 14.1, 3.6 Hz, 1H), 1.43 (m, 1H), 1.32 (dq, *J* = 12.8, 3.8 Hz, 1H), 1.17 (td, *J* = 13.5, 4.0 Hz, 1H), 1.12–1.03 (complex m, 2H), 1.03–0.95 (complex m, 2H), 0.92 (s, 3H), 0.90–0.84 (complex m, 7H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	120.6, 54.9, 54.4, 41.7, 39.5, 38.1, 36.3, 33.5, 33.3, 32.4, 21.8, 21.6, 20.9, 18.7, 15.1, 14.2.
MS (EI) m/z	233 (M^+), 219, 218, 123, 109, 95, 87, 82, 81.
$[\alpha]_D^{20}$	negligible (c 0.7, $CHCl_3$).

2-((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)acetonitrile



171

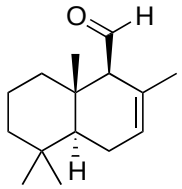
To a suspension of KCN (1.4 g, 21.5 mmol) in DMF (2 mL) was added drimenyl mesylate (**160**) (401 mg, 1.33 mmol) in DMF (3 mL). The resulting slurry was stirred at 70 °C for 18 h before the crude material was filtered through a silica plug eluting with 30 % EtOAc:hexane to yield a yellow oil (262 mg). Purification via automated gradient flash

column chromatography (silica; 0 → 12 % EtOAc:hexane) provided the title compound as a colourless oil (54 mg, 0.23 mmol, 17% yield). The spectroscopic data obtained was consistent with data reported in the literature.[330]

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.59 (m, 1H), 2.50 (dd, *J* = 16.9, 4.0 Hz, 1H), 2.27 (dd, *J* = 16.8, 7.0 Hz, 1H), 2.21 (m, 1H), 2.05 (m, 1H), 1.91 (m, 1H), 1.87–1.78 (complex m, 4H), 1.63–1.42 (complex m, 3H), 1.27–1.14 (complex m, 2H),1.06 (dt, *J* = 12.8, 4.5 Hz, 1H), 0.92 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 131.2, 125.0, 121.0, 51.6, 49.7, 41.9, 39.6, 36.5, 33.2, 32.9, 23.5, 21.9, 21.6, 18.6, 15.0, 13.8.

Drimenal



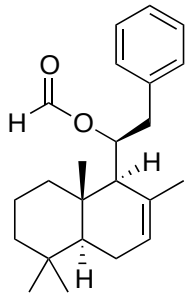
155

To a solution of (–)-drimenol (**118**) (160 mg, 0.72 mmol) at 0 °C in CH_2Cl_2 (5 mL) was added Dess–Martin periodinane (350 mg, 0.83 mmol) slowly. The reaction was warmed to rt and stirred for 15 min. Hexanes (10 mL) was added and the crude reaction mixture purified on a silica plug eluting with 60% CH_2Cl_2:hexanes to provide the title compound as a colourless oil (148 mg, 0.67 mmol, 93% yield). The spectroscopic data obtained was consistent with data reported in the literature.[273]

^{1}H NMR (600 MHz, $CDCl_3$): δ 9.62 (d, *J* = 5.1 Hz, 1H), 5.62 (m, 1H), 2.52 (s, 1H), 2.00 (m, 1H), 1.90 (m, 1H), 1.60 (dq, *J* = 13.3, 2.5 Hz, 1H), 1.55 (m, 3H), 1.47 (m, 1H), 1.40–1.35 (complex m, 2H), 1.25–1.10 (complex m, 3H), 1.08 (dd, *J* = 12.2, 4.7 Hz, 1H), 1.00 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 127.8, 125.5, 67.6, 49.1, 42.0, 40.4, 37.0, 33.2, 33.0, 23.7, 22.1, 21.6, 18.3, 15.7.

(S)-2-phenyl-1-((1S,4aS,8aS)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)ethyl formate



174

To a solution of drimenal (**155**) (50 mg, 0.28 mmol) in dry THF (5 mL) at 0 °C under N_2 was added benzylmagnesium chloride (700 µL, 2 M in THF, 1.4 mmol, excess). The reaction was stirred for 18 h before sat. NH_4Cl was added (5 mL) and the reaction extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield a colourless oil (88 mg). Purification via automated gradient flash column chromatography (silica; 0 → 20 % EtOAc/hexane) provided the title compound as a white amorphous solid (35 mg, 0.10 mmol, 36% yield).

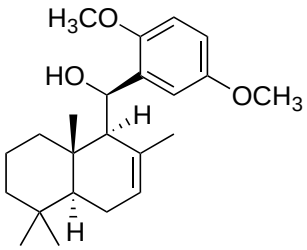
HRMS Found $[M+Na]^+$ 363.2298, $C_{23}H_{32}O_2Na$ requires M^+ 335.2295.

ν_{max} (NaCl): 3027, 2948, 2923, 2866, 2848, 1721 (C=O), 1455, 1389, 1173, 700 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.33–7.19 (complex m, 5H), 5.59 (m, 1H), 5.43 (dd, J = 9.9, 5.3 Hz, 1H), 3.13 (dd, *J* = 13.0, 5.3 Hz, 1H), 2.99 (dd, *J* = 13.0, 9.9 Hz, 1H), 2.02 (m, 3H), 2.00 (br s, 1H), 1.93 (m, 1H), 1.85 (m, 1H), 1.52 (m, 1H), 1.39 (dq, *J* = 13.8, 3.3 Hz, 1H), 1.32–1.21 (complex m, 2H), 1.02–1.94 (complex m, 2H), 0.83 (s, 3H), 0.80 (s, 3H), 0.73 (s, 3H), 0.18 (td, *J* = 13.3, 3.6 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$): δ	160.9, 137.4, 132.0, 129.9, 128.5, 126.7, 125.8, 72.8, 54.2, 49.7, 42.1, 41.8, 39.4, 36.6, 33.3, 32.8, 24.6, 23.3, 22.2, 18.5, 13.9.
$[\alpha]_D^{20}$	–46 (c 0.75, $CHCl_3$).

(*R*)-(2,5-dimethoxyphenyl)((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methanol



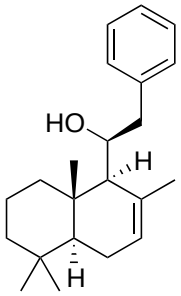
177

To Mg ribbon (200 mg) under N_2 was added a small crystal of I_2 which was allowed to sit for 5 min. Dry THF (5 mL) was added, followed by dropwise addition of 2-bromo-1,4-dimethoxybenzene (1.0 g, 4.61 mmol). The flask was passively cooled to rt before sitting for 10 min. The resulting Grignard reagent in THF was added slowly to a solution of drimenal (**155**) (190 mg, 0.86 mmol) in dry THF (5 ml) at 0 °C under N_2. The reaction was warmed to rt before being stirred for 1 h. Sat. NH_4Cl (20 mL) was added and the reaction extracted with CH_2Cl_2 (3 x 20 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield a colourless oil (509 mg). Purification via automated gradient flash column chromatography (silica; 0 → 12 % EtOAc:hexane) provided the title compound as a colourless oil (290 mg, 0.81 mmol, 94% yield)

HRMS	Found M^+ 358.2505, $C_{23}H_{34}O_3$ requires M^+ 358.2508.
ν_{max} (NaCl):	3469 (br, -OH), 2924, 2865, 2847, 1493, 1465, 1441, 1387, 1274, 1218, 1179, 1051, 1028, 835, 804, 757, 729 cm^{-1}.
^{1}H NMR (600 MHz, $CDCl_3$): δ	7.26 (d, *J* = 2.9 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 1H), 6.73 (dd, *J* = 2.9, 8.8 Hz, 1H), 5.60 (m, 1H), 5.25 (s, 1H), 3.79 (s, 3H), 2.65 (s, 1H), 2.15 (m, 1H), 1.98 (m, 2H), 1.65 (qt, *J* = 13.8, 3.0 Hz, 1H), 1.56 (m, 3H), 1.52 (m,

	1H), 1.48 (m, 1H), 1.33–1.21 (complex m, 3H), 1.14 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	153.3, 150.2, 135.3, 133.2, 126.7, 114.5, 111.5, 110.9, 67.5, 58.3, 55.8, 55.2, 50.2, 42.3, 40.0, 37.8, 33.6, 33.1, 24.8, 23.5, 22.5, 19.1, 15.4.
MS (EI) m/z	358 (M^+), 340, 325, 311, 265, 217, 205, 191, 176, 167, 151, 137, 123, 107, 95, 81.
$[\alpha]_D^{20}$	−15 (c 2.0, $CHCl_3$).

(*R*)-2-phenyl-1-((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)ethanol



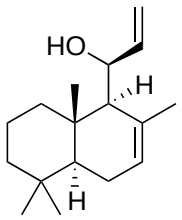
178

To a solution of drimenal (**155**) (37 mg, 0.17 mmol) in dry THF (5 mL) at 0 °C under N_2 was added benzylmagnesium chloride (600 µL, 2 M in THF, 1.2 mmol, excess). The reaction was stirred for 18 h before sat. NH_4Cl was added (15 mL) and the reaction extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield a colourless oil (200 mg, containing by-products from the reagent). Purification via automated gradient flash column chromatography (silica; 0 → 50 % EtOAc:hexane) provided the title compound as a colourless oil (38 mg, 0.12 mmol, 71% yield)

HRMS	Found $[M+Na]^+$ 335.2346, $C_{22}H_{32}ONa$ requires M^+ 335.2345.

ν_{max} (NaCl):	3589, 3483 (br, -OH), 3026, 2947, 2922, 2903, 2863, 2848, 1495, 1453, 1441, 1388, 1365, 1212, 1116, 1081, 1055, 1035, 837, 752, 719, 700 cm^{-1}.
^{1}H NMR (400 MHz, $CDCl_3$): δ	7.40 (m, 1H), 7.34 (t, 7.2 Hz, 2H), 7.26 (t, 7.2 Hz, 2H), 5.66 (m, 1H), 4.18 (t, *J* = 7.7 Hz, 1H), 3.16 (dd, *J* = 13.3, 8.9 Hz, 1H), 2.84 (dd, *J* = 13.4, 5.9 Hz, 1H), 2.04 (s, 3H), 2.03–1.87 (complex m, 3H), 1.75 (d, *J* = 13.0 Hz, 1H), 1.53 (m, 1H), 1.43–1.36 (complex m, 2H), 1.15–1.08 (complex m, 2H), 0.96 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.65 (dt, *J* = 13.3, 3.0 Hz, 1H).
^{13}C NMR (150 MHz, $CDCl_3$): δ	139.4, 132.5, 129.6, 128.5, 126.5, 126.4, 71.0, 58.1, 49.9, 45.6, 42.1, 39.8, 36.9, 33.4, 32.9, 25.0, 23.4, 22.3, 18.7, 14.4.
$[\alpha]_D^{20}$	+32 (c 0.75, $CHCl_3$).

(*S*)-1-((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)prop-2-en-1-ol



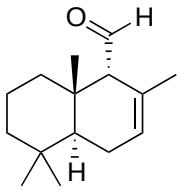
179

To a solution of drimenal (**155**) (50 mg, 0.23 mmol) in dry THF (4 mL) at 0 °C under N_2 was added vinylmagnesium bromide (800 µL, 0.8M in THF, 0.64 mmol, excess). The reaction was stirred for 18 h before sat. NH_4Cl was added (15 mL) and the reaction extracted with CH_2Cl_2 (3 x 15 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield a colourless oil (64 mg). Purification via automated gradient flash column chromatography (silica; 0 → 10 % EtOAc:hexane) provided the title compound as a colourless oil (44 mg, 0.20 mmol, 87% yield)

HRMS	Found M^+ 248.2137, $C_{17}H_{28}O$ requires M^+ 248.2140].

ν_{max} (NaCl):	3465 (br, -OH), 2949, 2923, 2865, 2847, 1455, 1441, 1388, 1365, 1134, 1052, 987, 915, 836 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ	6.10 (ddd, *J* = 17.3, 10.6, 4.7 Hz, 1H), 5.61 (m, 1H), 5.22 (dt, *J* = 17.3, 1.7 Hz, 1H), 5.06 (ddd, *J* = 10.6, 2.0, 1.5 Hz, 1H), 4.55 (m, 1H), 2.07 (s, 1H), 2.00–1.90 (complex m, 2H), 1.78 (m, 3H), 1.60 (qt, *J* = 13.6, 3.2 Hz, 1H), 1.52–1.41 (complex m, 2H), 1.24–1.14 (complex m, 2H), 1.05–0.99 (complex m, 4H), 0.93 (s, 3H), 0.89 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	144.4, 132.3, 126.5, 112.8, 70.0, 60.8, 50.0, 42.1, 40.3, 36.9, 33.4, 32.9, 24.3, 23.4, 22.3, 18.8, 15.0.
MS (EI) m/z	248 (M^+), 193, 192, 177, 149, 135, 123, 121, 110, 109, 107, 97, 95, 81.
$[\alpha]_D^{20}$	−22 (c 1.75, $CHCl_3$).

9-Epidrimenal



180

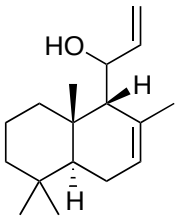
To a solution of 9-epidrimenol (**117**) (34 mg, 0.15 mmol) at 0 °C in CH_2Cl_2 (5 mL) was added Dess–Martin periodinane (100 mg, 0.24 mmol) slowly. The reaction was warmed to rt and stirred for 30 min. Hexanes (10 mL) was added and the crude reaction mixture purified on a silica plug eluting with 60% CH_2Cl_2:hexanes to provide the title compound as a colourless oil (28 mg, 0.13 mmol, 87 % yield). Used without full characterisation.

^1H NMR (400 MHz, $CDCl_3$): δ	9.61 (d, *J* = 5.3 Hz, 1H), 5.77 (m, 1H), 2.29–2.19 (complex m, 2H), 1.98 (m, 1H) 1.71 (dd, *J* = 11.8, 5.2 Hz, 1H), 1.68–1.56 (complex m, 4H), 1.55–1.44

(complex m, 2H), 1.33–1.15 (complex m, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ — 202.5, 126.6, 126.4, 68.3, 44.6, 42.4, 37.9, 35.9, 33.01, 32.97, 24.2, 22.4, 22.0, 21.1, 18.5.

1-((1R,4aS,8aS)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)prop-2-en-1-ol



181

To a solution of 9-epidrimenal (**180**) (28 mg, 0.13 mmol) in dry THF (5 mL) at 0 °C under N_2 was added vinylmagnesium bromide (500 µL, 0.8M in THF, 0.40 mmol, excess). The reaction was stirred for 72 h before sat. NH_4Cl was added (15 mL) and the reaction extracted with CH_2Cl_2 (3 x 15 mL). The combined organic fractions were dried ($MgSO_4$), filtered and evaporated to yield a colourless oil (43 mg) Purification via automated gradient flash column chromatography (silica; 0 → 10 % EtOAc/hexane) provided the title compound as a colourless oil (7 mg, 0.028 mmol, 22% yield)

HRMS — Found M^+ 248.2137, $C_{17}H_{28}O$ requires M^+ 248.2140.

ν_{max} (NaCl): — 3497 (br, -OH), 2938, 2909, 1462, 1388, 1379, 1365, 1124, 1111, 1048, 992, 917, 831 cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$): δ — 5.95 (ddd, *J* = 17.1, 10.5, 4.1 Hz, 1H), 5.73 (m, 1H), 5.28 (dt, *J* = 17.1, 1.9 Hz, 1H), 5.06 (dt, *J* = 10.5, 1.9 Hz, 1H), 4.53 (m, 1H), 2.11 (m, 1H), 1.88 (m, 1H), 1.78 (dd, *J* = 11.4, 6.0 Hz, 1H), 1.73–1.65 (complex m, 6H), 1.51 (m, 1H), 1.45 (m, 1H), 1.27–1.19 (complex m, 2H), 0.91 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H).

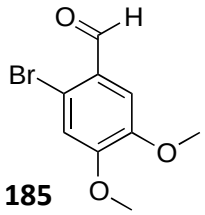
^{13}C NMR (150 MHz, $CDCl_3$): δ 144.1, 131.0, 126.9, 112.1, 68.5, 60.6, 42.6, 42.1, 36.9, 35.9, 33.1, 32.8, 25.6, 24.7, 22.2, 21.8, 18.8.

MS (EI) m/z 248 (M^+), 192, 191, 177, 149, 135, 121, 109, 107, 95, 87, 82.

$[\alpha]_D^{20}$ −143 (c 0.35, $CHCl_3$).

Indole synthesis

6-Bromoveratraldehyde



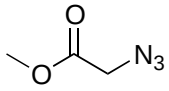
185

To veratraldehyde (**184**) (3,4-dimethoxybenzaldehyde, 10.0 g, 60.2 mmol) in MeOH (250 mL) was added bromine (3.2 mL, 62 mmol, 1.1eq). The reaction was stirred under air at rt for 2 h. The MeOH was evaporated under reduced pressure and the remaining residue dissolved in CH_2Cl_2 (150 mL), and washed with saturated sodium thiosulfate (200 mL) and brine (200 mL). The resulting organic fraction was dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure. NMR analysis showed residual starting material, so the material was resubjected to identical reaction conditions with a further 0.5 mL of bromine. Subsequent work up provided the title compound as a white amorphous solid (15.0 g, 61.2 mmol, >99% yield). Used without further purification. The spectroscopic data obtained was consistent with data reported in the literature.[331]

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.18 (s, 1H), 7.41 (s, 1H), 3.95 (s, 3H), 3.91 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 190.9, 154.7, 149.1, 126.7, 120.5, 115.6, 110.6, 56.6, 56.3.

Methyl 2-azidoacetate



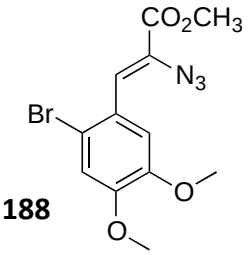
187

Sodium azide (4.2 g, 64.6 mmol) was added to methyl 2-bromoacetate (**186**) (3.0 mL, 4.86 g, 31.8 mmol) in acetone:water (3:1, 80 mL). The mixture was stirred in air at rt for 30 min before being diluted with water (100 mL), and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic fractions were dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to provide the title compound as a colourless oil (3.66 g, 31.8 mmol, >99% yield). Used without further purification. The spectroscopic data obtained was consistent with data reported in the literature.[332]

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 2H), 3.80 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9, 52.7, 50.4.

(*Z*)-Methyl 2-azido-3-(2-bromo-4,5-dimethoxyphenyl)acrylate



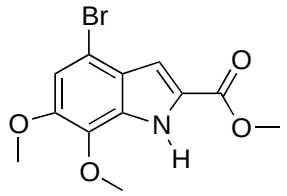
188

Methyl 2-azidoacetate (**187**) (3.0 g, 26.1 mmol) and 6-bromoveratraldehyde (**185**) (2.56 g, 10.4 mmol) were combined with dry MeOH (15 mL), and the resulting suspension cooled to -8 °C in an ice/salt bath. To this suspension was added a solution of sodium methoxide (sodium metal (620 mg, 26.97 mmol) in MeOH (15 mL)). The suspension was stirred at -8 °C for 10 mins, before dry THF was added until the majority of the starting materials had dissolved. The resulting solution was stirred at -8 °C for 90 min, before the flask was sealed and warmed to 4 °C for 16 h (without stirring). The resulting suspension of fine needles in a yellow solution was then poured onto ice cold saturated NH_4Cl solution (100 mL), and the solid collected by filtration on a Buchner funnel. The pale-yellow solid was washed with ice-cold water (2 x 50 mL), then collected by dissolution in CH_2Cl_2. The CH_2Cl_2 was dried ($MgSO_4$), filtered, and the solvent removed under reduced

pressure to provide the title compound as an off-white amorphous solid (2.01 g, 5.9 mmol, 57 % yield) used without further purification.

HRMS	Found M^+ 341.0000, $C_{12}H_{12}N_3O_4{}^{79}Br$ requires M^+ 341.0011.
ν_{max} (NaCl):	2106, 1701, 1501, 1433, 1387, 1265, 1209, 1167, 862, 806, 758 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ	7.84 (s, 1H), 7.23 (s, 1H), 7.06 (s, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	164.1, 150.4, 150.0, 125.22, 125.21, 123.8, 117.4, 115.5, 113.4, 56.30, 56.27, 53.2.
MS (EI) m/z	343 (^{81}Br-M^+), 341 (^{79}Br-M^+), 300, 298, 283, 281, 268, 266, 256, 254, 235, 159, 144, 132, 102, 59.

Methyl 4-bromo-6,7-dimethoxy-1*H*-indole-2-carboxylate



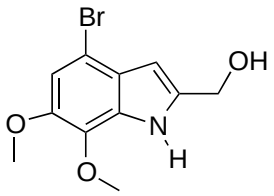
189

Azidoacrylate **188** (2.01 g, 5.9 mmol) was refluxed under N_2 in dry *m*-xylene for 1 hr. The mixture turned bright yellow, and was evaporated to dryness under reduced pressure to yield a yellow oil. Purification via automated gradient flash chromatography (silica; 0 → 30 % EtOAc:hexane) provided the title compound as an amorphous white solid (962 mg, 2.8 mmol, 47 % yield)

HRMS	Found M^+ 312.9953, $C_{12}H_{12}NO_4{}^{79}Br$ requires M^+ 312.9950.
ν_{max} (NaCl):	3322, 2938, 2837, 1705, 1628, 1508, 1441, 1321, 1258, 1206, 1130, 1067, 827, 750 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ	9.10 (br s, 1H, N-H), 7.16 (d, *J* = 2.4 Hz, 1H), 7.07 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	162.1, 149.4, 133.9, 131.9, 127.5, 124.8, 113.1, 109.8, 109.5, 61.2, 57.5, 52.3.
MS (EI) m/z	315 (^{81}Br-M^+), 313 (^{79}Br-M^+), 300, 298, 283, 281, 268, 266, 254, 252, 240, 238, 159, 144, 120, 118, 87, 84, 82.

(4-Bromo-6,7-dimethoxy-1*H*-indol-2-yl)methanol



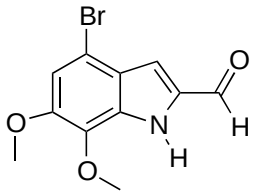
190

Methyl 4-bromo-6,7-dimethoxy-1*H*-indole-2-carboxylate (**189**) (962 mg, 2.8 mmol) was added to a suspension of $LiAlH_4$ (200 mg, 5.3 mmol) in *t*-butyl methyl ether and stirred at 35 °C for 30 min. The reaction mixture was then cooled to 0 °C, before being quenched by the subsequent addition of water (200 µL), NaOH (1 M aq., 200 µL), and water (600 µL), and stirred for 15 min. The reaction mixture was then dried ($MgSO_4$), and filtered through Celite™ with CH_2Cl_2 (100 mL). The solvents were removed under reduced pressure to provide the title compound as a white amorphous solid (843 mg, 2.9 mmol, >99 % yield). Used without further purification.

HRMS	Found M^+ 284.9990, $C_{11}H_{12}NO_3{}^{79}Br$ requires M^+ 285.0001.
ν_{max} (NaCl):	3426, 1632, 1512, 1422, 1333, 1248, 1229, 1123, 1015, 789 cm^{-1}.
^{1}H NMR (400 MHz, DMSO-d6): δ	6.99 (s, 1H), 6.17 (s, 1H), 5.14 (t, *J* = 6.0 Hz , 1H, O-H), 4.55 (d, *J* = 5.6 Hz, 2H), 3.84 (s, 3H), 3.82 (s, 3H).
^{13}C NMR (100 MHz, DMSO-d6): δ	146.5, 141.5, 133.9, 130.3, 125.0, 110.4, 106.0, 98.8, 60.5, 57.4, 56.6.

MS (EI) m/z	287 (^{81}Br-M$^+$), 285 (^{79}Br-M$^+$), 272, 270, 254, 252, 226, 224, 117, 99, 87, 84, 82, 79.

4-bromo-6,7-dimethoxy-1*H*-indole-2-carbaldehyde

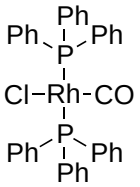


192

(4-Bromo-6,7-dimethoxy-1H-indol-2-yl)methanol (**190**) (843 mg, 2.9 mmol) was suspended in CH_2Cl_2 (15mL), and slowly added (~20 min) to a suspension of Dess-Martin periodinane (2.4 g, 5.7 mmol) and $NaHCO_3$ (1.8 g, 21.4 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The resulting suspension was stirred for 45 min at 0 °C before water (50 mL) was added, followed by solid sodium metabisulfite until the mixture stopped producing bubbles. The organic fraction was separated, and extracted further with CH_2Cl_2 (2 x 10 mL). The combined organic phase was washed with saturated $NaHCO_3$ (50 mL), before being dried ($MgSO_4$), filtered, and evaporated under reduced pressure to yield a crude brown solid. The crude material was filtered through a plug of silica eluting with EtOAc:hexanes (3:7, 100 mL) to provide the title compound as a pale yellow amorphous solid (796 mg, 2.8 mmol, 97 % yield).

HRMS	Found M$^+$ 282.9838, $C_{11}H_{10}NO_3{}^{79}Br$ requires M$^+$ 282.9844.
ν_{max} (NaCl):	3298, 1663, 1622, 1508, 1321, 1244, 1136 cm^{-1}.
^{1}H NMR (400 MHz, $CDCl_3$): δ	9.79 (s, 1H), 9.17 (br s, 1H, *N*-H), 7.20 (d, *J* = 2.2 Hz, 1H), 7.10 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	181.4, 150.6, 136.2, 133.9, 133.2, 124.7, 115.2, 113.4, 110.9, 61.2, 57.5.
MS (EI) m/z	285 (^{81}Br-M$^+$), 283 (^{79}Br-M$^+$), 270, 268, 161, 146, 133, 118, 105, 87.

Chlorocarbonylbis(triphenylphosphine)rhodium(I)

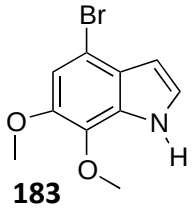


193

Rhodium(III) chloride (0.41 g, 1.91 mmol) was added to a solution of water (0.2 mL, 10.2 mmol) in DMF (15 mL) in a sealed tube. The suspension was refluxed for 1 h, sonicated for 30 min at rt, then refluxed again for 1.5 h until the solid dissolved and the solution turned yellow. The solution was filtered to remove residual solid material, and PPh_3 (0.97 g, 3.7 mmol) was added slowly, liberating CO from the complex, and forming a solid yellow crystalline product. The suspension was poured onto ice cold water (50 mL), and the supernatant decanted away. The resulting solid was washed with hot diethyl ether (4 x 5 mL), and dried under reduced pressure to provide the title compound as a fine yellow powder (910 mg, 1.32 mmol, 69 %). The spectroscopic data obtained was consistent with data reported in the literature.[333]

ν_{max} (NaCl):	1973, 1481, 1435, 1096, 745, 692 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ	7.78–7.69 (m, 12H), 7.35–7.35 (m, 18H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	134.9 (t, *J* = 6.3 Hz), 133.1 (t, *J* = 28.1 Hz), 130.2 (s), 128.3 (t, *J* = 4.9 Hz) (carbonyl not observed by ^{13}C NMR).
^{31}P NMR (162 MHz, $CDCl_3$): δ	29.0 (d, *J* = 127.2 Hz).

4-bromo-6,7-dimethoxyindole



183

To freshly distilled *m*-xylene (15 mL) was added chlorocarbonylbis(triphenylphosphine)rhodium(I) (**193**) (17 mg, 0.025 mmol) and the

suspension stirred at 80 °C until the complex had completely dissolved to form a pale yellow solution (~15 min). dppp (20 mg, 0.048 mmol) was added and the solution turned bright yellow. After 15 mins, a fine yellow precipitate was formed (*cis*-chlorocarbonyl(dppp)rhodium(I)). 4-bromo-6,7-dimethoxyindole-2-carboxaldehyde (**92**, 60 mg, 0.21 mmol) was added and the suspension was heated to reflux. After 18 h stirring at reflux, the resulting light-brown solution was evaporated to dryness under reduced pressure, dissolved in EtOAc:hexanes 1:1, and filtered through a plug of silica eluting with EtOAc:hexanes (1:1, 100 mL) to yield 63 mg of crude material. Purification via automated gradient flash chromatography (silica; 0 → 60 % EtOAc/hexane) provided the title compound as an off-white amorphous solid (22 mg, 0.086 mmol, 41 %).

HRMS	Found M^+ 254.9886, $C_{10}H_{10}NO_2{}^{79}Br$ requires M^+ 254.9895.
ν_{max} (NaCl):	3354, 2938, 1504, 1410, 1337, 1294, 1233, 1082, 949, 882, 826, 772, 729 cm^{-1}.
^1H NMR (400 MHz, $CDCl_3$): δ	8.33 (br s, 1H, N-H), 7.18 (t, *J* = 2.5 Hz, 1H), 7.02 (s, 1H), 6.49 (t, *J* = 2.5 Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	147.4, 134.2, 130.6, 125.2, 124.4, 111.8, 107.8, 103.3, 61.1, 57.8.
MS (EI) m/z	257 (^{81}Br-M^+), 255 (^{79}Br-M^+), 242, 240, 227, 133, 122, 121, 120, 119, 118, 117.

X-ray crystallography data

X-ray data was collected and processed by Dr. Nathan Kilah at the University of Tasmania and at the Australian Synchrotron.

X-ray diffraction data for compounds **12**, **14**, **54**, **116**, **118**, **121**, **139**, **168**, and **174** were collected with monochromated Cu Kα radiation (λ = 1.54178 Å) from an Incoatec IμS Cu microsource on a Bruker D8 Quest, equipped with a PHOTON 100 CMOS detector. Single crystals were mounted on nylon loops with viscous immersion oil, and placed into a chilled nitrogen stream (Oxford Cryosystems Cobra), and the data were collected at 100(2) K with the APEX3 software. Series of ϕ and ω scans were performed to a

maximum resolution of 0.79 Å. X-ray diffraction data for compounds **15**, **122**, and **137** were collected on the MX1 beamline of the Australian Synchrotron,[334,335] with silicon double crystal monochromated radiation (λ = 0.71073 Å). All structures were solved using charge flipping methods in SUPERFLIP,[336] or Intrinsic Phasing with SHELXT,[337] and refined using full-matrix least-squares on F^2 with SHELXL within the OLEX2 suite.[338,339] Comparisons with the known stereochemistry of the polygodial starting material, **12,** refinement of the Flack parameter,[340] and analysis of the Bayesian statistics of the Bijvoet pairs in PLATON,[341,342] were used to assign absolute configuration of the stereogenic carbon centers. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms on carbon were generally visible in the diffraction map, but were included at calculated positions and ride on the atoms to which they are attached. Hydrogen atoms on the oxygen atoms, with the exception of those in **122**, were visible in the difference map and their positions were refined. The alcohol hydrogen atoms in **122** were not easily identified, and a combination of electron density and anticipated hydrogen bonding patterns were used to place the hydrogen atoms. Molecular graphics were produced with OLEX2.[339] Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (1570561–1570569, 1571233).

Compound #	**12**	**14**	**15**
formula	$C_{15}H_{22}O_2$	$C_{15}H_{22}O_2$	$C_{15}H_{26}O_2$
formula weight	234.32	234.32	238.36
crystal habit, color	needle, colorless	plate, colorless	plate, colorless
crystal size, mm	0.05 x 0.10 x 0.20	0.06 x 0.28 x 0.28	0.01 x 0.04 x 0.04
temperature, K	100	100	100
crystal system	orthorhombic	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
a, Å	7.5624(2)	6.6675(2)	7.3930(15)
b, Å	15.3693(4)	6.7401(2)	10.397(2)
c, Å	22.9240(6)	29.3269(8)	18.193(4)
β, °			95.29(3)
Volume, Å^3	2664.43(12)	1317.94(7)	1392.4(5)
Z	8	4	4
Z'	2	1	2
D_{calc} g.cm^{-3}	1.168	1.181	1.137
radiation	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)	Mo Kα (λ = 0.71073)
μ, mm^{-1}	0.592	0.598	0.073
GOF	1.066	1.097	1.044
independent reflns	4597	2608	7719
obs reflns ($I > 2.0\sigma(I)$), R_{int}	4480 (0.0263)	2602 (0.0231)	6601 (0.0698)
2θ range, °	6.924–132.386	12.07–144.798	4.518–63.452
final *R*, *wR* ($I > 2.0\sigma(I)$)	0.0266, 0.0665	0.0313, 0.0819	0.0553, 0.1309
R, *wR* (all data)	0.0275, 0.0659	0.0313, 0.0820	0.651, 0.1389
largest diff peak and hole, e Å^{-3}	0.263, -0.146	0.198, -0.176	0.330, –0.261
Flack parameter	–0.03(3)	0.05(2)	0.4(5)[b]
CCDC No.	1570561	1570562	1570563

Compound #	**116**	**121**	**122**
formula	$C_{15}H_{26}O_2$	$C_{15}H_{28}O_2$	$C_{15}H_{28}O_2$
formula weight	238.36	240.37	240.37
crystal habit, color	plate, colorless	rod, colorless	plate, colorless
crystal size, mm	0.01 x 0.10 x 0.12	0.10 x 0.10 x 0.30	0.005 x 0.05 x 0.05
temperature, K	100	100	100
crystal system	orthorhombic	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$	$C222_1$
a, Å	6.4338(2)	7.0240(2)	8.3986(17)
b, Å	13.4695(3)	8.5288(2)	12.780(3)
c, Å	32.2541(8)	22.6967(5)	26.660(5)
β, °			
Volume, Å^3	2795.14(13)	1359.68(6)	2861.6(10)
Z	8	4	8
Z'	2	1	1
D_{calc} g.cm^{-3}	1.133	1.174	1.116
radiation	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)	Mo Kα (λ = 0.71073)
μ, mm^{-1}	0.565	0.581	0.071
GOF	1.056	1.088	1.075
independent reflns	4794	2687	3938
obs reflns ($I > 2.0\sigma(I)$), R_{int}	4131 (0.0565)	2599 (0.0351)	3772 (0.0443)
2θ range, °	7.112–132.832	7.790–144.234	7.072–63.400
final *R*, *wR* ($I > 2.0\sigma(I)$)	0.0460, 0.0992	0.0313, 0.0800	0.0544, 0.1416
R, *wR* (all data)	0.0584, 0.1042	0.0324, 0.0808	0.0568, 0.1433
largest diff peak and hole, e Å^{-3}	0.203, -0.223	0.157, –0.205	0.285, -0.226
Flack parameter	0.14(12)	0.02(6)	0.0(3)[b]
CCDC No.	1570564	1570565	1570566

Compound #	**118**	**168**	**174**
formula	$C_{15}H_{26}O$	$C_{30}H_{54}O_3S$	$C_{23}H_{32}O_2$
formula weight	222.36	494.79	340.48
crystal habit, color	plate, colorless	needle, colorless	plate, colorless
crystal size, mm	0.18 x 0.18 x 0.18	0.04 x 0.04 x 0.20	0.06 x 0.10 x 0.10
temperature, K	100	100	100
crystal system	monoclinic	orthorhombic	orthorhombic
space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
a, Å	7.3963(2)	21.9131(5)	7.3487(2)
b, Å	22.4145(5)	7.3410(2)	12.9019(3)
c, Å	12.2947(2)	7.3410(2)	20.5850(5)
β, °	93.7600(10)		
Volume, Å^3	2033.88(8)	2777.09(12)	1951.71 (8)
Z	6	4	4
Z'	3	1	1
D_{calc} g.cm^{-3}	1.089	1.183	1.159
radiation	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)
μ, mm^{-1}	0.0493	1.241	0.553
GOF	1.045	1.161	1.062
independent reflns	8286	5925	3410
obs reflns ($I > 2.0\sigma(I)$), R_{int}	7996 (0.0321)	5844 (0.0741)	3260 (0.0297)
2θ range, °	7.206–149.274	6.518–158.546	8.088–133.318
final *R*, *wR* ($I > 2.0\sigma(I)$)	0.0339, 0.0897	0.0538, 0.1371	0.0316, 0 0786
R, *wR* (all data)	0.0352, 0.0907	0.0544, 0.1375	0.0334, 0.0801
largest diff peak and hole, e Å^{-3}	0.377, –0.195	0.379, –0.420	0.143, –0.167
Flack parameter	0.16(7)[b]	0.212(7)[b]	0.11(10)[b]
CCDC No.	1570567	1570568	1570569

Compound #	**139**	**54**	**137**
formula	$C_{19}H_{30}O_2$	$C_{15}H_{26}O_3$	$C_{15}H_{22}N_2$
formula weight	290.43	254.36	230.35
crystal habit, color	plate, colorless	plate, colorless	plate, colorless
crystal size, mm	0.05 x 0.1 x 0.3	0.2 x 0.12 x 0.04	0.1 x 0.1 x 0.05
temperature, K	100	100	100
crystal system	monoclinic	monoclinic	monoclinic
space group	*I*2	$P2_1$	$P2_1$
a, Å	12.6828(6)	11.1931(3)	6.5540(14)
b, Å	8.2885(3)	9.2227(3)	29.644(6)
c, Å	16.2674(10)	14.9319(4)	7.3710(15)
β, °	99.441(2)	102.9282(13)	115.95(3)
Volume, Å^3	1686.89(14)	1502.36(8)	1287.7(6)
Z	4	4	4
Z'	1	2	2
D_{calc} g.cm^{-3}	1.144	1.125	1.188
radiation	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)	Mo Kα (λ = 0.71073)
μ, mm^{-1}	0.554	0.61	0.07
GOF	1.061	1.052	1.126
independent reflns	2768	6031	5395
obs reflns ($I > 2.0\sigma(I)$), R_{int}	2583 (0.049)	5726 (0.057)	5272 (0.027)
2θ range, °	8.218–133.242	6.072–149.070	6.734–55.918
final *R*, *wR* ($I > 2.0\sigma(I)$)	0.0376, 0.0927	0.0404, 0.1021	0.0420, 0.1249
R, *wR* (all data)	0.0409, 0.0953	0.0427, 0.1041	0.0429, 0.1254
largest diff peak and hole, e Å^{-3}	0.169, –0.188	0.38, –0.23	0.301, –0.200
Flack parameter	–0.12(18)	0.18(7)	–0.4(7)
CCDC No.	1571233	N/A	N/A

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